ICI institute for chemical immunology

*Interview*

# "GLYCO IS THE NEXT WAVE"

**CAROLYN BERTOZZI**
*Professor of Chemical and Systems Biology*
*Stanford University, CA, USA*

**For chemist Carolyn Bertozzi it was always clear that studying glycans will help us to better understand biology. Recently, more and more biologists are warming up to that notion. "We are on the brink of an exponential growth in glycobiology research."**

*In a recent paper in Nature Reviews Drug Discovery, you refer to glycans as 'the dark matter of biology'. Are they really that unknown and mysterious?*

"Well, I would say it is getting lighter, things are really accelerating in glycoscience."

*In what way?*

"Look at the speed in which we have gone from a viral genome sequence to a vaccine; it is no accident that we were able to move so fast. We were powered by a century of technology development, largely from physics and chemistry. I think that glycoscience is now starting to enjoy a similar boost from technology advances and we will start to see an exponential increase in glycobiology discoveries and innovations."

*What are the main drivers behind this technology boost?*

"Advancements in mass spectrometry have made a huge difference. We can now determine the structures of glycans as well as where they are attached to protein scaffolds. Of course, there is still plenty of room for technology growth, ▶

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but if you consider where we were ten years ago and what we can do now, it is a big change. Another great advance has been the synthesis of glycans. That used to be extremely challenging, but now thanks to the work of chemists like Peter Seeberger, automated glycan synthesis is on the rise. Also, biocatalytic synthesis of glycans using enzymes is taking off, take for example the work of Xi Chen. Being able to make glycans on scale has really boosted efforts to define their biological functions."

"But these technologies have not yet been democratized. We don't have a full-fledge glyco-serving biotech reagent industry yet, like we have for genomics and proteomics. For example, I don't need to make my own oligonucleotides anymore, I just order them. That is what we also need for glycoscience, a glyco-industry, and that requires a critical mass of customers. Now that more and more biologists are stumbling into glycoscience from completely different angles, that market is developing."

*Stumbling?*

"Let's say you're an immunologist who wants to study how a T-cell migrates into the tumor microenvironment and what it does there, then you start with CRISPR screen and glycogenes pop up at the top of your hit list. It could even be that the top-10 is exclusively made up of glycogenes; genes involved in the biosynthesis of glycans. Biologists in this situation often reach out to people like me to get help interpreting such results."

**"The glyco-immune axis offers new targets"**

*That is a recent development?*

"For most researchers, glycobiology was seen as too complicated, too many unknowns, so they were tempted to brushed aside any observations that pointed toward a role for glycans in their pathway of interest. But now it is becoming easier to study glycans and the importance of glycobiology in human health and disease is increasingly acknowledged. I hope that in twenty years from now, glycoscience is no longer defined as a separate field, but has been fully integrated into 'biology'."

*What kind of biological questions can we now tackle, using these new technological possibilities?*

"Thanks to the advances in mass spectrometry, we can now study how glycans change in disease. The glycome is very rich in biological information; it is sensitive to the cell's physiology and can reveal something about the presence of malignancies or about stress or inflammation or cell damage. The use of glycoprofiling - comparing glycosignatures from cancer patients and healthy subjects - is already being evaluated in clinical trials. We have started a company that is doing glycoproteomic profiling of blood samples, for example."

*You have been active in this area already for a long time. Is it still exciting or is it perhaps becoming a bit more of the same?*

"Every new scientific area follows a progression that resembles a logarithmic curve. First you have a long lag phase where nothing much seems to happen due to the need for new technology development, then there is an exponential increase in activity during the log phase and finally you reach a plateau. I feel that now, glycoscience is moving from the lag to the log phase. We are on the brink of an exponential growth in glycobiology research."

**"Advancements in mass spectrometry have made a huge difference"**

*And where will we see the most activity?*

"For the next five years, I would say that the link between glycoscience and cancer immunology is a very visible topic. A major recent discovery is that tumor-associated glycosylation patterns can suppress immune cells, enabling tumor persistence and growth. This glyco-immune axis offers new targets for immune therapy. I like to think that glyco is the next big biopharma wave and we are ready to ride it."

"In twenty years from now, neuroscience will be the frontier for glycobiology, I predict. The brain has a unique glycome that is very different from all the other organs in the body. Why that is? Well, all that circuitry has to be organized and when Nature needs more complexity in biological regulation, she often deploys the glycome."

*For over a year now, we all have been operating in a very restricted world. In spite of all the downsides, are there perhaps also things that we should keep doing?*

"One very positive thing is that we are reducing travel time, thanks to all the options we now have for interacting at a distance. I will definitely keep on using that, for example, to invite guest lectures to my group meetings, without them having to travel great distances."

*When the world opens up again, what will you do first?*

"I just want to go to the lab, walk around, talk to everyone, just hang out together. I really miss that. As for my personal life, my kids are really looking forward to having birthday parties again. I think throwing a party will be one of the first things we do." ■





# PRECISION IN ANTICANCER VACCINES

**Leiden biotech company ISA Pharmaceuticals develops therapeutic vaccines against HPV, hepatitis B, melanoma and also Sars-CoV2. Currently, clinical trials are running in late-stage neck and head cancer and cervical cancer caused by HPV. "In immunology, your science needs to be very precise," stresses co-founder Kees Melief.**

It's an exciting time for ISA Pharmaceuticals. Their lead product, anticancer vaccine ISA101b, is tested in a randomized phase II clinical trial in conjunction with a checkpoint blocking monoclonal antibody. A successful trial will mean a breakthrough for ISA's technology. Chief Scientific Officer Cornelis ('Kees') Melief: "In the past fifteen years, many declared anticancer vaccines a failure. I'm convinced anticancer vaccines will be a success. You just need to be very precise, using the right target, the right platform, the right adjuvant and, if necessary, the right co-treatment."

ISA Pharmaceuticals is a Leiden-based biotech company with 35 employees. Emeritus professor Melief, who has a long career in academic science: "I was involved in early studies with CD19 and CD20-binding monoclonal antibodies. We were stunned by their antitumor effects, but back then filing for patents was not something an academic researcher would do. Afterwards, I promised myself to grab the next opportunity to bring a valuable technology to the market."

## SLP-technology

That opportunity is the SLP-technology. SLP stands for Synthetic Long Peptide. SLPs are a kind of prodrug. SLP therapies consist of 10-15 peptides with a length of around 30 amino acids. These 'fragments' are identical to parts of viral proteins or proteins overexpressed in tumor cells. After injection they are presented by dendritic cells to T-cells thus initiating an immune response. An adjuvant called AMPLIVANT completes ISA's SLP-therapy by boosting dendritic cell activity.
When the company started in 2004, monotherapy in cervical cancer was the prime focus. Yet, for successful therapy in late-stage cancer, SLPs need to be combined with other therapy like checkpoint blockers. The vaccine currently in clinical trials, ISA101b, elicits an immune response against cells infected with human papillomavirus type-16. This virus is responsible for about half of all cervical cancers and 85 percent of HPV-positive head and neck cancers. ISA101b showed promising results in combination with anti-PD1 therapy in a small trial including 22 patients in late-stage head and neck cancer with no curative therapeutic options left. Overall survival was doubled from 9.1 months to 17.5 months. "That's a very positive result for this type of cancer," says Melief. "Moreover, ISA101b adds specificity to the therapy, but no toxicity."
ISA-products are based on thorough studies in immunology, he stresses. "We cooperate with academic research groups in fundamental research, with the universities of Leiden, Utrecht and Nijmegen. The details are important. We are actually the only company that performed a thorough clinical dose-response study for cancer vaccines. To me, that is essential knowledge."

## Covid variants

ISA Pharmaceuticals pipeline also holds SLP-vaccines against melanoma, hepatitis B and Sars-CoV2. "SLPs against melanoma take the technology a step further," explains Melief. "We are targeting human cancer-associated antigens instead of viruses." The SLP in ISA's Sars-CoV2 therapy are not only directed against the spike protein like current vaccines, but also against nuclear and membrane proteins. ISA's aim is developing a vaccine that protects against a broad spectrum of coronaviruses, which would include all variants of Sars-Cov2 and also emerging SARS or MERS-viruses. Melief: "Seeing how fast the virus mutates in India or Brazil, we need to be prepared as much as possible." ■

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# Studying a tumor cell's camouflage

## PhD project: Probing and harnessing tumor cell's glycocalyx to improve immunotherapy

**The sugar-rich covering on tumor cells probably shields them from the immune system. Using click chemistry PhD-students Daan Smits and Yuri Damen study this outermost layer and hope to use it against the malignant cells.**

Chemist Yuri Damen enjoys a synthetic challenge. "It requires creativity. You need to consider all kinds of routes that may lead to the compounds you want to make. Moreover, I like the 'cooking', the actual hands-on work in the laboratory." Making compounds for click-chemistry, like Damen is currently doing, may be called a true challenge. "It's always balancing. You need strain in the molecules to make sure they easily 'click', but too much strain will make them unstable or just impossible to make."
Damen 'cooks' for Daan Smits, his ICI PhD-partner at Radboud UMC, who uses Damen's compounds to study the interaction of T-cells from the immune system with tumor cells. Smits: "Tumor cells often have a thicker outer layer than healthy cells: a thick glycocalyx." Like the name suggests this glycocalyx (calyx is Greek for bark) contains many sugar groups. Smits: "Our hypothesis is that this helps tumor cells to evade the immune system. The thick layer of glycoproteins physically hinder T-cells in recognizing tumor-specific antigens on the cell's surface."

### Faster

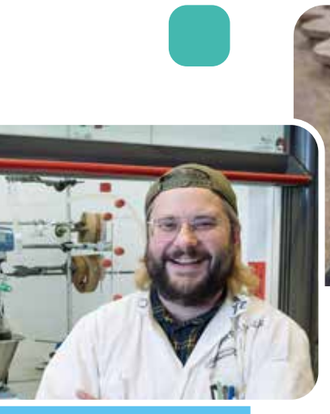
To unravel the exact role of the glycocalyx Smits studies the first interaction of T-cells and tumor cells under the microscope. Click chemistry is used to vary the thickness and composition of the glycocalyx. Damen: "You can feed tumor cells sugars containing azide-groups. These will appear on the surface and can be used to click on all kinds of stuff." 'Stuff' like fluorescent labels, additional glycans, but also cytokines or immunoregulatory proteins. "And when you add a specific linker, you may release these compounds again."
A world of possibilities, but in practice the toolkit in click chemistry is still limited. The chemistry needs to work in a natural environment, which means in water and with as little as possible (toxic) effects on the living cells. Smits: "And to witness the first contact, the chemistry needs to work fast. Currently Yuri is working at our request on a faster fluorescent probe."

*"They come up with ideas we wouldn't think about and the other way around"*

Damen also intends to attach linkers for click chemistry to compounds that attract T-cells. Smits: "When we can click these onto the glycocalyx and then slowly release them, a concentration gradient may lure T-cells towards tumor cells. It's far away, but nevertheless it's an inspiring idea that this might one day advance immune therapy."

### Working together apart

Very early in his studies, Damen was convinced that a PhD was what he wanted. Smits, however, wasn't sure that academic research was 'his thing'. After finishing his MSc, he took a temporary job as a technician in a university laboratory. After a few months, he started longing for his own project. "That you may actually discover new things yourself, I discovered that that is pretty cool."
Damen and Smits talk to each other "when necessary" - they have clear, separate tasks, they both agree. Yet, they also stress the importance of working together. Damen: "When we meet with the whole team to solve a problem, they come up with ideas we wouldn't think about and the other way around. It just really works well." ■



**Yuri Damen (left)**
Organic Chemistry, WUR
Floris van Delft group

**Daan Smits**
Cell Biology, Radboudumc
Peter Friedl group



PhD projects

# Mapping autoantibody diversity

## PhD project: Personalized profiling of patient autoantibodies linked to rheumatoid arthritis

**Not only the number of autoantibodies but also their N-glycosylation increases when the first symptoms of rheumatoid arthritis arise. PhD-students Eva Maria Stork and Danique van Rijswijck dive deep into this conspicuous phenomenon.**

"We want to study changes in autoantibodies of people with rheumatoid arthritis, from the pre-disease to disease state, and during treatment and flares," tells Eva Maria Stork, PhD-student at Leiden UMC. "Revealing the patterns may help rheumatologists prescribing medication more precisely - not too early, and not too late," adds her ICI PhD-partner Danique van Rijswijck, who works at Utrecht University.
The presence of a specific type of autoantibodies, the ACPAs – Anti-Citrullinated Protein Antibodies -, in blood plasma is a hallmark of rheumatoid arthritis (RA). Yet, disease-onset may still be years away. Research indicates that the onset of RA is also accompanied by an increase in N-glycosylation of the variable domain of the ACPAs. Therefore, variable domain glycosylation of ACPAs may be an important biomarker for RA. However, the role of the glycosylation is still a mystery.

### Strong tool

Van Rijswijck will study ACPAs using advanced mass spectrometry techniques and bioinformatics. She likes that the project combines two of her favorite disciplines: physics and biology. "Proteins are fascinating, and it is impressive which information we can get from them using mass spectrometry." Yet, antibodies are diverse and N-glycosylation makes them even more complex. "It is quite a challenge, but if we succeed, we will know more about RA *and* have developed a new research tool. Because we are developing an MS-based approach to sequence the secreted antibodies, which in the end can be used in a broad range of studies in immunology."

*"We are really focused on what is needed to help patients"*

Stork just handed over a first batch of monoclonal ACPAs to Van Rijswijck which she produced and purified. Stork holds a MSc in Molecular Biotechnology from Heidelberg University and moved to Leiden in the fall of 2020. "There are easier times to move abroad for a PhD then now with the pandemic, but luckily I already knew the environment from an internship in Leiden a few years ago." Stork will select patients and purify ACPAs in the various stages of the disease. She also studies the autoreactive B-cell receptors that precede the secreted ACPA. "Studies of the antibody repertoire are now limited to sequencing of the B-cell receptors. When we manage to also sequence the autoantibodies, we can directly compare the B-cell receptors and the secreted antibodies. This will allow us – for the first time – to directly evaluate whether the repertoire of memory B-cells reflects the ACPAs secreted to the plasma."
Van Rijswijck is puzzling which strategy could work best to analyze the ACPAs. "We cut off the constant domains as it are the variable regions that make up the autoantibody diversity. However, it's not clear yet whether it's also necessary to cleave off the N-glycans. First, I need to learn more about the behavior of ACPAs in the mass spectrometer."

### Hand-in-hand

To their regret Stork and Van Rijswijck haven't met yet 'in real life' because of the pandemic. Contact runs via app messages and an online meeting every two months. Van Rijswijck: "I really like the cooperation with Eva Maria. It connects my mass spectrometry work directly to the clinic. That makes the work more relevant to me and it feels like we are really focused on what is needed to help patients." Stork: "It's nice to work kind of hand-in-hand, and both our expertise are necessary to complete the project." ■

**Eva Maria Stork (left)**
Rheumatology, LUMC
Tom Huizinga/René Toes group

**Danique van Rijswijck**
Biomolecular Mass Spectrometry and Proteomics, UU
Albert Heck laboratory

# ICI PhD students after finishing their thesis

**The young doctors start their career. Which path do they choose and why? Laurent Paardekooper and Lianne Lelieveldt share their experiences.**

## “I really love working in the lab”

Laurent Paardekooper is sure: the party in the laboratory is not over yet. Doing research and experiments is actually pretty fun for him. “I have a passion for science and how it can improve our lives. I also believe in the importance of fundamental science, which is the fuel for successful translational and applied projects.” His wish came true when he started as a post-doctoral researcher in Maartje Huijber’s Neuroimmunology group at the Department of Human Genetics at Leiden University Medical Center (LUMC). He works on neuromuscular autoimmune diseases, in particular MuSK myasthenia gravis, a disease where non-inflammatory IgG4 antibodies attack the muscle protein MuSK. “My role is to investigate whether and how patient’s B cells deviate and how they came to the IgG4 isotype. Finally, I hope to find therapeutic targets.”
Paardekooper benefits the successful collaborations with scientists and students from different disciplines during his PhD. “It broadened my horizon and enhanced my creative thinking.” This multidisciplinary experience is expanding further on now, for instance by being introduced into completely new genetic research techniques such as single cell RNA sequencing. “I learn every day and I enjoy to roll up my sleeves in the lab and combining practical work with theoretical investigation.”
For the present, he is very pleased with his job. After this postdoc fellowship, he will consider next steps. Does he want to stay in academia or switch to the corporate world e.g., in the Bio Science Park across the street from his lab? Or perhaps becoming a policy adviser at NWO or the Ministry of Health, Welfare and Sport. “I do not know yet. For the time being, I have plenty of challenges here.” ■

## “This job fits me like a glove”

“Traveling is my passion, therefore I went on a world trip before applying for a job,” Lianne Lelieveldt explains.
The trip was not necessary to reflect on her professional ambitions, because she had no doubts about that. “My drive to understand biological processes and their interaction with small molecules has convinced me to pursue a career in the field of pharmaceuticals.” She found the ideal job at Synaffix, specialized in antibody drug conjugate (ADC) technology platforms. A young and ambitious start-up company that aims to become the preferred partner for pharma and biotech companies focused on the development of these complex biological therapeutics.
“I am given the opportunity to firstly enrich my knowledge and experience. Starting at the R&D department, where research allows a lot of freedom and creativity, I work on ADC technology in general. Soon, however, I will combine this to partner projects, which implies custom work aimed at specific applications. I am looking forward to this, because in addition to scientific research, it requests social skills. That will be exciting and challenging.”
Lelieveldt looks back with pleasure on the ICI duo PhD project. “I am a bio-organic chemist and learned a lot from my partner Hendy Kristyanto, an immunologist, who proved to be an excellent teacher and taught me a lot about biological processes.” In the long run Lianne aspires a management position in which she may lead a team of researchers. As yet, however, she is sure: “In my current position there is still a lot to learn for me. I am happy with this job and get plenty of energy from my work.” ■

▼ *After successfully having defended his PhD thesis ‘Oxygen shapes the early immune response’ (October 2019) Laurent Paardekooper started his career as postdoctoral researcher at LUMC.*

▼ *Lianne Lelieveldt defended her PhD thesis ‘Chemical strategies for antigen-selective targeting of autoreactive B Cells’ (May 2019) and, after several months of travelling, she started as a scientific researcher at Synaffix.*



# Look who’s talking

**Understanding what goes on in a population, whether human or cellular, requires intimate knowledge of who is in touch with whom, what they are talking about and how they respond to the newly acquired information. A recent paper in Science presents a clever combination of existing techniques to tackle cellular cross-talk in the central nervous system.**

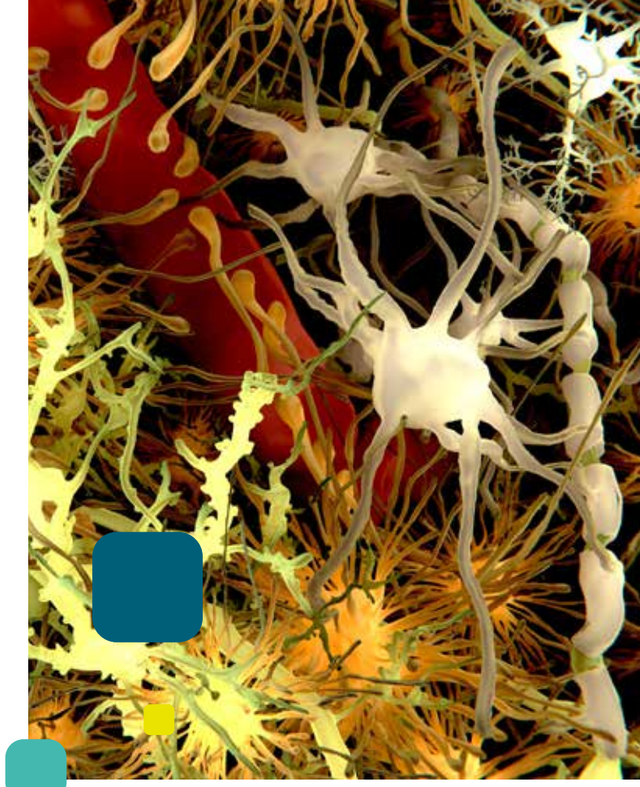
Single cell techniques have revolutionized cell biology research and have proven to be extremely valuable to unravel the intricate cellular networks that make up the immune system. But plenty more challenges remain, for example related to mapping cellular cross-talk *in vivo*, in real time and to get a grip on the location of these local communication networks in both healthy and diseased tissues. In Science, Iain Clark, Cristina Gutiérrez-Vázquez, Michael Wheeler (all of Harvard Medical School) and co-workers present a new approach to dissecting *in vivo* cellular cross-talk between astrocytes and microglia called RABID-seq. It combines a viral barcode tracing with single-cell RNA sequencing. “A very clever combination,” comments Joost Snijder, assistant professor at Utrecht University.

### Particular glycoprotein

The RABID-seq paper was brought to his attention by postdoc Matti Pronker, a structural biologist who studies receptors in the CNS. “In this paper, it is all about a glycoprotein that the rabies virus used to bind those receptors,” Snijder explains. “We study the rabies virus as a pathogen and this particular glycoprotein is very interesting to us because the rabies virus uses it to bind neurons and to jump from cell to cell using synaptic connections. That capability can also be employed to track direct contact between cells.”
To turn the pathogen into a tracking device, the Harvard-team removed the receptor-binding glycoprotein from the rabies virus. In the mouse model used, which in this case was a model for multiple sclerosis, they incorporated an alternative glycoprotein in the mouse astrocytes. Snijder: “The modified virus enters the astrocytes, binds to the alternative glycoprotein which allows the virus to replicate and spread to neighboring cells that have synaptic connections to the astrocyte. Once the virus enters another cell, it cannot bind and replicate anymore and the process stops. This way, you can deduce which cells are in direct contact with an astrocyte.”

### Like a barcode

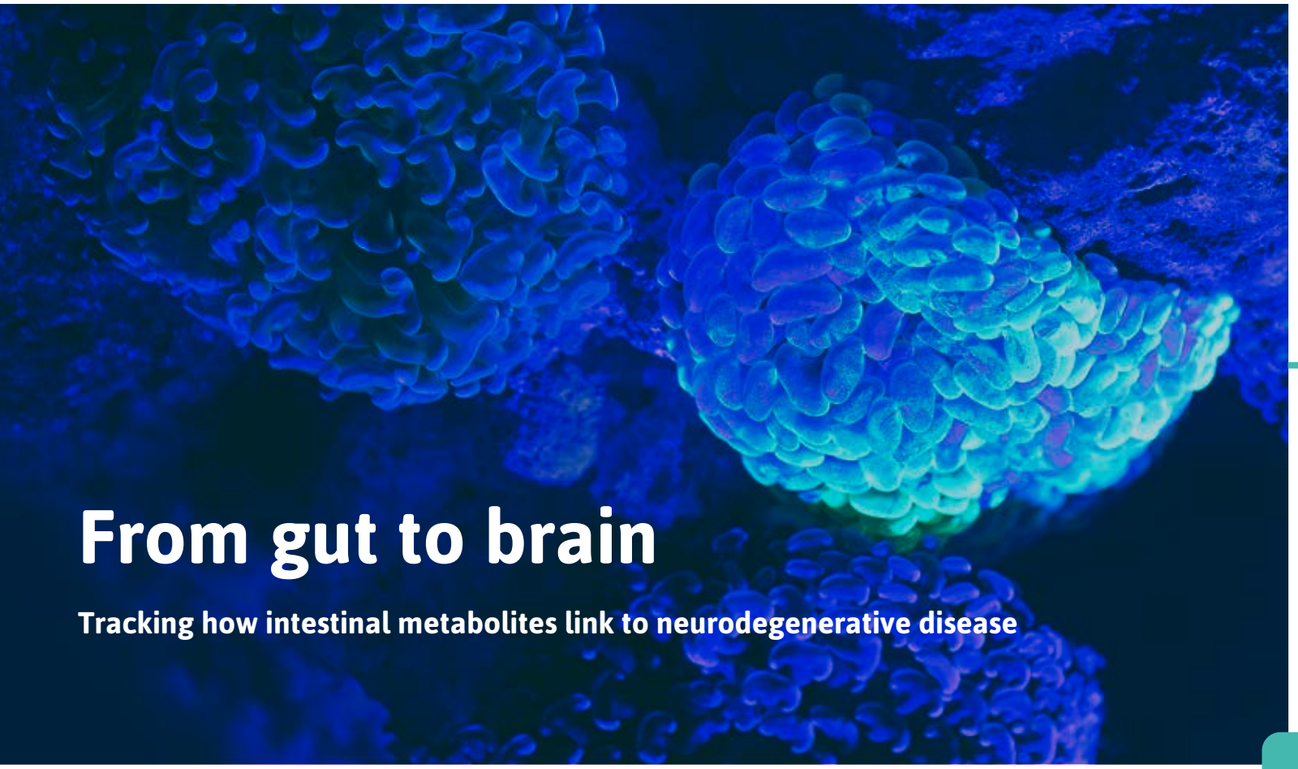
To zoom in on the close contacts of individual astrocytes, each viral particle contains a small, unique sequence that acts like a barcode. “By ‘reading’ the barcodes in each cell, you can group cells together into small, localized networks of cross-talking cells.” Using the rabies virus as a tool in such ‘connectomics’ measurements has been done before, says Snijder. “But what is really smart here, is that they combine the connectomics approach with single-cell RNA sequencing. That generates a really deep insight into what happens on the molecular level in each cell that is part of a cross-talk network. It provides expression profiles of microglia that are in contact with astrocytes. It really is a beautiful technique to dissect the molecular basis of interactions between different cell types.”
The relevance for ICI is clear, according to Snijder. “There is a shared interest in microglia because of their role in neuroinflammation, which is a common theme in the new neuro-immunochemistry program. This new technique offers much needed opportunities to study the CNS of which we are still only seeing the tip of the iceberg.” ■



▲ *Main cells in the central nervous system (CNS) comprise: neurons (yellow), astrocytes (orange), oligodendrocytes (gray) and microglia (white). Cell-cell interactions control the physiology and pathology of the CNS. Advanced techniques are needed to study such mutual cell-cell communication. The discussed Science paper presents a new approach, called RABID-seq, to dissecting in vivo cellular cross-talk between astrocytes and microglia.*

### Reference

Clark IC *et al*. Barcoded viral tracing of single-cell interactions in central nervous system inflammation, Science (April, 2021), Vol 372, Issue 6540, eabf 1230; DOI: 10.1126/science.abf1230



# From gut to brain

## Tracking how intestinal metabolites link to neurodegenerative disease

**ICI is expanding its scientific reach. The newly initiated neuro-immunochemistry program encompasses five projects that each address a different aspect of the neuro-immune axis; the interplay between the immune system and neurological processes. One of these projects involves a collaboration between the research groups of Aletta Kraneveld, professor of Interdisciplinary Translational Pharmacology and Celia Berkers, professor of Metabolomics, both based at Utrecht University. Together, they aim to tackle the role of the neuro-immune axis in Parkinson's disease and for that, they have chosen a rather surprising starting point: the gut.**

To be more precise, the project focuses on the metabolism of the immune cells of the enteric nervous system (ENS), the autonomously operating nerve system located in the gut. The ENS actively communicates and interacts with the central nervous system, creating the so-called gut-brain axis. Berkers: "The ENS is the gateway to the brain." And this axis is highly relevant to Parkinson's disease, says Kraneveld. "It has been known for a long time that many patients who suffer from a brain-related disease, also experience serious intestinal problems. And for Parkinson's, we know that in most cases these problems were already present years before the disease manifested itself. We want to understand how processes in the gut affect neurological processes in the brain." And whatever happens in the gut is directly linked to the immune system. After all, our intestines are the largest exterior part of our body, as Kraneveld puts it. So, there is the gut-brain axis, comprising two nervous systems, and there is the immune system. Quite a puzzle, considering the overwhelming amount of cell types, proteins, metabolites, tissues and pathways involved. Not to mention the variety of bacteria that inhabit the gut; part of the microbiome. But that is exactly where the search for new insight will start. Kraneveld: "To keep it all manageable, we are concentrating on the metabolites secreted by the gut bacteria and how they affect intestinal neuro-immune interactions."

*"The enteric nervous system is the gateway to the brain"*

### Prion-like behavior

The ENS has its own immune cells, the glia cells, which operate like macrophages and play a role similar to that of the microglia in the brain. "Gut bacteria generate a huge number of different metabolites," says Berkers. "But which metabolites activate the glia cells and what happens after activation? How do the glia cells process these metabolites to generate new signals? Which cells are subsequently affected by these signals? What are the pathways involved



Collaboration

and can we link those to functional output? We want to understand this on the molecular level to map the whole track from metabolite via glia activation to the effects in the enteric nervous system." Even though there is still much unknown about this track, there is ample evidence for a direct relationship between immunometabolic processes in the gut and the clinical hallmarks of Parkinson's. The dopaminergic

*"We are expanding our research field to the neuro-immune axis"*

neurons located in the *substantia nigra*, which is the brain's control center for movement, are the first to get damaged in Parkinson's, leading to the characteristic motor impairments. "The damage in the dopaminergic neurons is caused by an accumulation of the protein alpha-synuclein. But we see that same accumulation in the gut, in the neurons that are part of the ENS," Kraneveld explains. "One of the leading hypotheses is that these alpha-synuclein accumulations in the gut neurons start to behave like prions and spread through the vagus nerve towards the brainstem, where they encounter the vulnerable dopaminergic neurons in the substantia nigra. These neurons are rather fragile and they get easily damaged, leading to the motor problems that are usually a first indication of Parkinson's Disease."

### Balancing phenotypes

The context and questions are clear, but how are these translated to hands-on studies in the lab? Berkers: "Our aim is to create an *in vitro* model of the ENS in which we will culture glia cells and neurons together, so we can measure how changes in glia metabolism, initiated by bacterial metabolites, affect the metabolism and function of other cells, particularly the neurons." The emphasis on metabolism is a no brainer. "We know that metabolism is key to understanding immune cells, activation of immune cells always goes hand in hand with changes in the cell's metabolism. And bacterial metabolites are important signals that cause these changes." But there are so many different metabolites to consider and so many different bacteria that produce them. Where to start? Are there particularly relevant bacteria and/or metabolites when it comes to Parkinson's? "Yes, there are. The microbiome of patients shows significantly enhanced levels of gram-negative bacteria. That is still a very large group, but it is a start," says Kraneveld. "We also know of certain bacterial products that are more present in Parkinson's, including lipopolysaccharide, LPS, which is well known for its link to inflammation. LPS binds to TLR4 and that receptor is present on the glia cell surface." Another interesting clue is the lack of short-chain fatty acids. These compounds are important to the energy management of immune cells, but they also play a role in cell's balance between phenotypes. "Glia cell can have an inflammatory or regulatory phenotype," explains Kraneveld. "It is common for immune cells to exhibit an inflammatory phenotype and that is needed to fight pathogens, but that status should not persist. Going back to a 'neutral' status is crucial, but in many diseases this balance is disturbed."

### Human models

What will this project – if it all works out – contribute to the field? "The new models we are building using human cells and combining different cell types, that is quite innovative," says Berkers. "That will generate opportunities for other applications in research related to intestinal and neurological diseases, and we will gain more insights into the gut-brain axis. Maybe we can, in time, also include the vagus nerve to study the communication towards the brain." And in terms of technology development? "We have to develop new analytical approaches. The short-chain fatty acids are new to us, so we have to figure out how to analyze those profiles. And of course, there is the challenge of measuring multiple cell types simultaneously, while at the same time keeping track of what goes on in each individual cell type. We have not done that before, but it is the way to go. Metabolism is not an isolated process; it is influenced by what happens in a cell's environment." ■

## New ICI theme

The recently initiated neuro-immunochemistry program encompasses the interplay between the immune system and neurological processes. Sander van Kasteren, ICI Junior Board Member, explains: "The immune system of the brain remains largely unknown. A serious a problem, because the interactions of the brain-resident immune cells play a major role in the development of both the healthy brain and brain-related diseases, several of which affect young people disproportionately. A group that tends to get overlooked when it comes to medical research funding. We strongly feel that ICI's chemical biology approach could be useful here and through close collaborations between chemists, immunologists and brain researchers, we will start designing the toolkit to address major questions in the field. We hope that the new projects, like the one by Celia and Aletta, enable a new cross-over research field."

Highlights

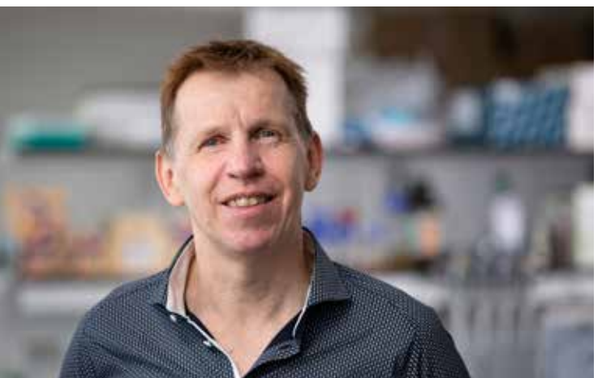
# News


### ICI Conference 2021

Last March 19th, almost 200 participants attended the first online edition of the ICI conference 2021. The conference was live streamed from a professional studio at the Van Nelle Factory in Rotterdam. Presenter Esther Thole guided the program, addressed the participants, introduced the speakers and moderated the Q/A session after each lecture.
Lectures were presented by renowned scientists from the Netherlands and abroad. The latter were easier to contract as they could stay at home without affecting their usually overcrowded schedules. Also, the youngest generation of ICI researchers, recently started PhD students, had been included in the program. Earlier they were challenged to write an abstract about their research during a course 'science communication'. Three winners of the abstract competition were chosen to pitch their research at the conference. Despite lacking face-to-face talks due to limitation of online presentations and discussions, the professional approach was highly appreciated by participants and speakers. In all probability ICI's future conferences will comprise a hybrid version with the best of the two: a physical meeting where participants meet and speak each other, combined with a live stream display and virtual contributions by renowned international scientists.

### Jeantet-Collen Prize for translational medicine

Ton Schumacher, group leader Molecular Oncology & Immunology at the Netherlands Cancer Institute and Professor of Immunology at Leiden University and Leiden UMC, is to receive the Jeantet-Collen Prize for Translational Medicine 2021. He is receiving this prize together with Jerome Galon, research director at the Institut National de la Santé et de la Recherche Médicale.
Both scientists are awarded for their pioneering contributions to the understanding of how the immune system can recognize cancer cells and to the understanding of the mechanism of action of new cancer immunotherapies. They will use the prize (€ 500K) to further understand the immune microenvironment during tumor transition. For instance, to develop new diagnostic and therapeutic technologies by predicting which tumor antigens will be recognized by T-cells. Next to this award Schumacher has recently also been elected as '2021 Fellow of the American Association for Cancer Research Academy' for his vast contributions to characterizing antitumor immunity.



### Ceppellini Award

The European Federation for Immunogenetics has honored Professor Sjaak Neefjes, head of the department of Cell and Chemical Biology at Leiden UMC, with the Ceppellini Award. The prize is awarded to a scientist that has made substantial contributions to the field of immunogenetics. Each year, the honored scientist is invited to present the Ceppellini lecture at the annual European Federation for Immunogenetics (EFI).

April 21th, Neefjes delivered an online presentation of this lecture entitled 'My MHC life and beyond: a fascination of the ins and outs of antigen presentation'. "My work dates back to when I was a PhD student. At the time, it was unclear how the adaptive immune system recognized virus-infected cells," Neefjes explains. As a chemist interested in cell biology, he applied non-traditional technologies from the field of immunology to explore how MHC molecules processed antigens and elicited anti-viral immune responses. "In doing so, I discovered many of the steps that occur during these complex pathways."
Building on these fundamental studies, Neefjes pioneered techniques that enabled the visualization of several cellular life processes. As a result, he uncovered novel mechanisms for drug action and later developed several clinical applications for infectious diseases, autoimmune diseases and cancer, including radioimmunotherapy for the treatment of specific tumors.

### Jeannette de Wolf

As of October 2020, Jeannette de Wolf is project manager at the Leiden Institute for Chemical Research. In this position she also runs ICI's financial project management. She likes her job very well: "As a chemist, I feel closely involved in scientific research and it is fantastic to experience research progress close up." Together with Pauline Hoftijzer (project assistant) and Daphne van Elsland (project manager), she completes ICI's back-office team.

### Vidi grant

Annemarthe van der Veen (ICI Junior Board Member and head of the Molecular Biology of Innate Immunity group, LUMC) has been awarded by an NWO Vidi grant (€ 800K) for her project 'Inflammation without infection'. If the immune system no longer distinguishes between body's own cells and microbial cells such as viruses, an inflammatory reaction may take place without there being an infection. Van der Veen's group is going to use the grant to investigate such sterile inflammations.

### PNAS Cozarelli prize

The PNAS article that resulted last year from the ICI PhD project (Dennis Wander and Sabina van der Zanden) has now been awarded with the PNAS Cozzarelli Prize in the Biomedical Sciences field. The research team, guided by Hermen Overkleeft, found a way to reduce the side effects of the anticancer drug doxorubicin without losing its effectiveness.

# Recent publications

Hammink R, Weiden J, Voerman D, Popelier C, Eggermont LJ, Schluck M, Figdor CG, Verdoes M.
**Semiflexible Immunobrushes Induce Enhanced T cell Activation and Expansion**
ACS Appl. Mater. Interfaces 2021, 13, 16007-16018; doi: 10.1021/acsami.0c21994

Wörner TP, Bennett A, Habka S, Snijder J, Friese O, Powers T, Agbandje-McKenna M, Heck AJR.
**Adeno-associated virus capsid assembly is divergent and stochastic**
Nat Commun. 2021 Mar 12;12(1): 1642; doi: 10.1038/s41467-021-21935-5. PMID: 33712599

Andrian T, Bakkum T, van Elsland DM, Bos E, Koster AJ, Albertazzi L, van Kasteren SI, Pujals S.
**Super-resolution correlative light-electron microscopy using a click-chemistry approach for studying intracellular trafficking**
Methods Cell Biol. 2021,162, 303-331; doi: 10.1016/bs.mcb.2020.09.001.Epub 2020 Oct 16. PMID: 33707017

Van Dalen FJ, Bakkum T, van Leeuwen T, Groenewold M, Deu E, Koster AJ, van Kasteren SI, Verdoes M.
**Application of a Highly Selective Cathepsin S Two-step Activity-Based Probe in Multicolor Bio-Orthogonal Correlative Light-Electron Microscopy**
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Čaval T, Hecht ES, Tang W, Uy-Gomez M, Nichols A, Kil YJ, Sandoval W, Bern M, Heck AJR.
**The lysosomal endopeptidases Cathepsin D and L are selective and effective proteases for the middle-down characterization of antibodies**
FEBS J. 2021 Mar 13; doi: 10.1111/febs.15813. Online ahead of print. PMID: 33713388

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Front Immunol. 2021 Jan 14, 11:608466; doi: 10.3389/fimmu.2020.608466. eCollection 2020. PMID: 33519818

Wander DPA, van der Zanden SY, Vriends MBL, van Veen BC, Vlaming JGC, Bruyning T, Hansen T, van der Marel GA, Overkleeft HS, Neefjes JJC, Codée JDC.
**Synthetic (N,N-Dimethyl)doxorubicin Glycosyl Diastereomers to Dissect Modes of Action of Anthracycline Anticancer Drugs**
J Org Chem. 2021 Mar 30; doi: 10.1021/acs.joc.1c00220. Online ahead of print. PMID: 33783212

*Column*

# FULL TV-STUDIO ON-SITE



## SANDER VAN KASTEREN
*ICI JUNIOR BOARD MEMBER*

*Sander van Kasteren is associate professor at the Leiden Institute of Chemistry*


When I young, I used to be a bit of a heavy metal fan. In those days, there was one weekend more important than all others: the weekend of Dynamo Open Air. This festival was of such renown that all bands played there for free. The honor of playing was enough.
It was this festival that came to mind often whilst co-organizing this year's online edition of the ICI-conference. For good and bad reasons. Let me start with the latter: it was at this festival that I first encountered the trepidations that come with off-site contributions to a program. Metallica, the heavy metal behemoths, were meant to showcase their new album one year, via a satellite-linked Q&A session. However, due to the stormy weather that usually plagues Dutch festivals, the video screen had blown away, leaving said showcase the biggest dud of the festival.

This was our major fear for the ICI2021: that technical glitches would leave the conference dead in the water. We therefore ensured proper technical support for the proceedings. What we didn't realize, however, was what it would entail! The company Bourgonje spent the morning building up a full TV-studio on-site, which served as a broadcast hub. Nothing like the two flimsy bits of white sheet that Metallica were hoping to use at Dynamo.
The other inspiration that Dynamo Open Air offered, came from their programming; how they had always managed to get the best artists to our tiny country. We therefore decided to use the online element to our advantage and book speakers that had previously eluded us due to their phenomenally busy schedules.

The 'headliner' was a particular triumph for us: we had been trying to get Carolyn Bertozzi to speak at the ICI for the past 5 years, but her busy schedule had always prevented it. Her stature as the founder of chemical biology, and major innovator in the field of chemical immunology, made us feel she was the perfect fit for this audience. And thanks to Covid, we finally succeeded. And – due to the other lessons learned – the whole conference went by without any digital equivalent of a blown away video screen.
So, in future, we are thinking about keeping some of the good bits of this conference and perhaps attempt a hybrid version where we get some speakers to dial in and present on a screen, whilst other, more local, talent can be present on site. Or perhaps we should aim really big, and ask Metallica for the next edition. ■


## About ICI
The Institute for Chemical Immunology (ICI) is a Gravitation project, made possible by the Ministry of Education, Culture and Science, in collaboration with the Netherlands Organization for Scientific Research (NWO). ICI will define and exploit a new field termed chemical immunology and train a novel generation of interdisciplinary scientists. It aims to promote academic excellence. The ICI publishes twice-yearly the 'ICI Bulletin' featuring ICI research, education and other relevant developments. To (un)subscribe please send an mail to info@chemicalimmunology.nl.

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| Lay-out | SPRANQ, Alexander Kraaij |
| Photography | Laura Morton, Lennert Janssen, Erik van 't Hullenaar, Thijs Rooimans and Guy Ackermans |
| Printing | Bestenzet BV, Zoetermeer |



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