Interview

"TO TREAT NEUROPSYCHIATRIC DISORDERS, YOU NEED TO UNDERSTAND THE MOLECULAR CAUSES"

Institute for Chemical Neuroscience

MARIO VAN DER STELT *(RIGHT)*
Board-member

SANDER VAN KASTEREN
Workpackage leader

The Institute for Chemical Neuroscience kicks off in December. Several ICI members participate in the new institute which studies human brain tissue to discover the molecular causes of psychiatric disorders. An interview with iCNS board-member Mario van der Stelt and workpackage leader Sander van Kasteren of Leiden University. "The concept is the same as in ICI: chemists and biologists are working closely together to solve scientific problems."

There seems to be quite an overlap in ICI and iCNS members?

Mario van der Stelt (**MvdS**): "Yes, and that is not a coincidence. Within ICI, we ran a research project with Inge Huizinga of the Netherlands Institute for Neuroscience which has provided much of the groundwork for iCNS. A lot of young principal investigators from ICI join in, too. Kim Bonger, Monique Mulder and Martijn Verdoes, for example. You might say that iCNS has sprouted from ICI."
Sander van Kasteren (**SvK**): "The concept behind ICI and iCNS is the same: chemists and biologists are working closely together to solve scientific problems. In iCNS, the focus is not on immunology, but on unravelling the molecular origin of neuropsychiatric disorders such as depression, bipolar disorder and schizophrenia. Diseases that don't get as much attention as Alzheimer's or Parkinson's disease, but affect one in four, and also young people who still have a whole life ahead of them." ▶

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How will iCNS achieve this goal?

MvdS: "In psychiatry, diagnoses are made by ticking off symptom checklists in the DSM-5. There is no biological basis underlying the diagnoses. However, just like lung cancer can have different causes – different mutations in various cells – a condition such as schizophrenia or depression is likely to be highly heterogeneous too. To effectively treat such diseases, you need to understand those molecular causes."
"We will study the transcriptome, the proteome and the metabolome of cells in various regions of human brains donated by people with neuropsychiatric disorders to the Netherlands Brain Bank, and search for differences at the molecular level with brains of people without such problems. Using large language models that read through the medical records we want to link molecular characteristics to particular disorders and subgroups thereof."
SvK: "We are going to study brain cells in much detail. We will look for example into the interactions between neurons and microglia, the immune cells of the brain, to exactly understand changes. Standard techniques like creating a knock-out mouse are not informative in neuropsychiatric research. Thus, the challenge, for us as chemists, is: how do you get the same information without genetic modification? Within ICI we have already developed some methods that we can use."
MvdS: "To give an example: we have developed an activity-based histology method to visualize enzymatic activity in fixed and fresh-frozen human brain tissue at subcellular level. We will apply it in iCNS-projects."

Why focus on neurosciences? Tools developed in ICI can also be used for example in cancer research.

SvK: "For many patients with neuropsychiatric problems there is currently no effective medical therapy, and the pipelines of pharmaceutical companies are all but empty. Clinical experiments are currently underway with drugs such as ketamine and LSD. That is old stuff with many side effects. In my vision, neuropsychiatric disorders are developmental syndromes which often start in puberty. When we understand the molecular causes, we hope to find new drug targets, too. The current lack of new targets is mainly due an absence of suitable animal models in neuropsychiatry; there is no such thing as a 'depressed mouse'."
MvdS: "Some psychiatrists do not agree with our approach. They believe the mind cannot be contained in molecules. I don't agree. I'm especially interested in patients for which medication isn't helpful. What is different in their brains? Neuropsychiatric diseases are an important burden for people and society, I think we should invest much more in understanding what is happening in the brain at the molecular level."

"Neuropsychiatric disorders affect one in four, also young people"

Is there enough brain material available for your research?

MvdS: "There have been donor recruitment campaigns in the Netherlands before asking people with neuropsychiatric disorders to donate their brain to sciences after their death. The Netherlands Brain Bank already contains hundreds of such brains, and a considerable part of the iCNS budget goes to a new recruitment campaign. The current number is large enough to get an early view, but for sufficient statistical power the numbers need to be increased. We will start with studying the human brain to detect potential target proteins, and check and validate our hypotheses in advanced iPSCs [induced pluripotent stem cells, ed.] and animal models. We kind of 'reverse' the drug discovery process."

Will couples of PhD students work in iCNS, like in ICI?

SvK: "In ICI we coupled a chemist with an immunobiologist. We noticed, however, that the timing and balance in workload wasn't always optimal. In iCNS, we couple a chemist to two or more neurobiologists, and preferably the chemist will start somewhat earlier. Furthermore, we involve AI and data scientists and organoid specialists."

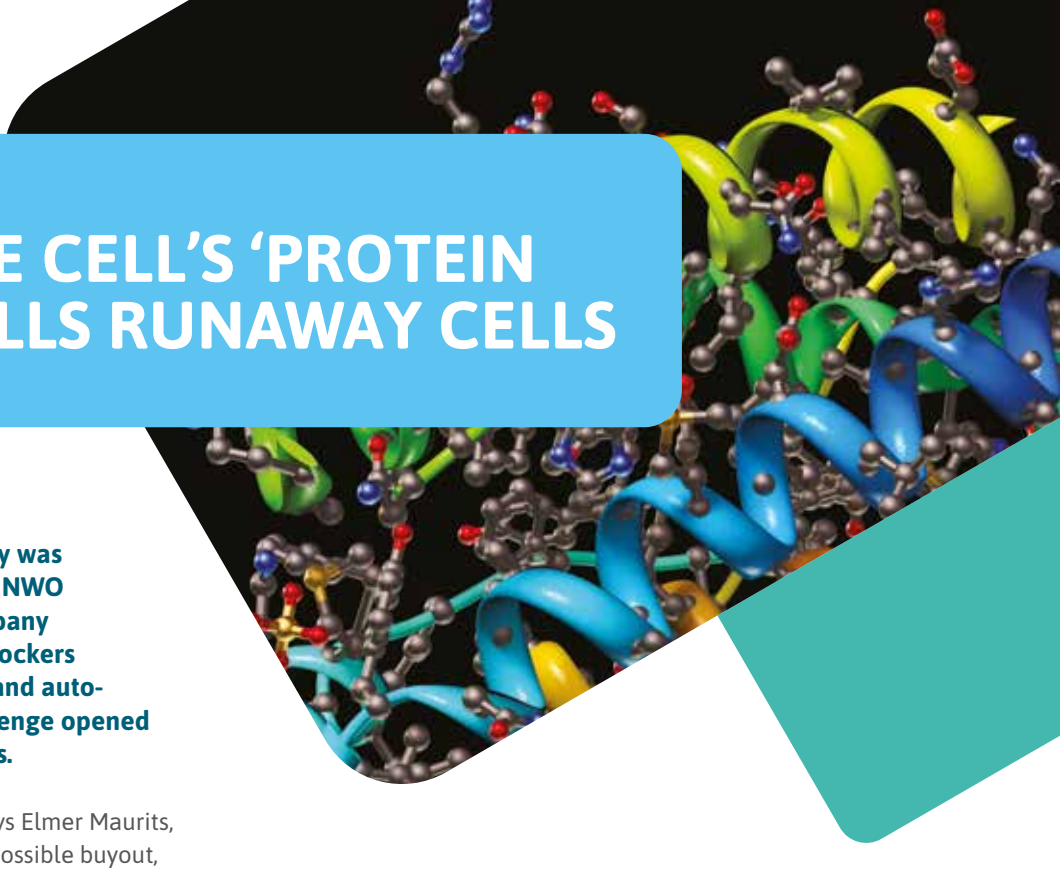
"Good communication is very important when you work at the interface of two disciplines"

MvdS: "There are also strong ties with Brainscapes, another gravitation program in neurosciences. For optimal cooperation, we are going to teach young chemists more about molecular neuroscience, too. I'm preparing a new course, here in Leiden."
SvK: "We know from ICI that good communication is very important when you work at the interface of two disciplines. I can get pretty confused in a conversation with a neuroscientist because of our different scientific language. Mario, however, has been working in this field for a long time. I foresee that he will be an important node in the iCNS community, a kind of translator."

What do you hope to achieve with iCNS?

MvdS: "I will be happy when we can explain to groups of patients what is causing their neuropsychiatric disorder in ten years, because we know which molecular processes in the brain are dysregulated. Of course, we hope to identify new targets for drug development, too."
SvK: "It's exciting to stand at the start of such a new, large project with lots of new collaborations. I feel that ICI has resulted in opening up a research field; I see more and more being published in chemical immunology and receive invitations for meetings also outside the Netherlands. I hope that iCNS has the same effect in the field of chemical neurosciences." ■

BLOCKING THE CELL'S 'PROTEIN SHREDDER' KILLS RUNAWAY CELLS



iProtics a spin-off of Leiden University was launched this year, after winning the NWO Venture Challenge in 2022. The company is developing immunoproteosome blockers as promising therapeutics in cancer and auto-immune diseases. "Winning the challenge opened many doors," says COO Elmer Maurits.

"I don't mind becoming a millionaire," says Elmer Maurits, co-founder of iProtics. However, after a possible buyout, he thinks he would immediately start a new company. "To me, business is foremost about bringing a good idea to the community, to patients. That's what I want to do."
To learn more about how to run a business, organic chemist Maurits followed the PLNT Leiden Venture Academy in 2022 during a postdoc. "The Academy is about personal development: do you really want to become an entrepreneur, and can you learn the necessary skills? I really enjoyed it. Also, because I act as the manager of the deathcore band Distant in which I play bass guitar."

Venture challenge winner

In the spring of 2022, Maurits also participated in the NWO Venture Challenge. A competition, held twice a year, between six teams with ambitions to start a business in life sciences, explains Chretien Herben, who developed the accelerator program. During two intensive boot camps of three days, the teams compose a solid business plan and pitch their ideas to potential investors. Herben: "We teach contesters to focus on their future product or service. Will there be sufficient demand? What about competitors in the field?"
The iProtics team consisted of professor in Chemical Biology Hermen Overkleeft, assistant professor Bobby Florea and PhD student Patrick Dekker. Maurits: "It was quite a revelation. We started out with a pitch focused on our scientific findings. At the end, the science was condensed in one slide, without any molecules."
iProtics won and spend a larger part of the 25.000-euro prize money to seek legal aid in license negotiations with Leiden University. With an impact grant of the same university, proof-of-principle could be established, leading to the official launch of iProtics in 2024. Maurits: "Currently, I'm responsible for the business development; Patrick focuses on the science."

Immunoproteosome

The startup develops therapeutics that block the immunoproteasome. "The proteosome is the protein shredder of a cell. It chops up redundant proteins to provide the cell with fresh amino acids to construct new proteins," explains Maurits. "When you block the proteosome, the supply of building materials falters and the cell dies."
The immunoproteosome is a proteosome variant that is produced under stress and is very active in runaway cells such as cancer cells. Maurits: "We can selectively block the immunoproteosome, which prevents many of the side effects of currently used proteosome blockers. That's our unique selling point." iProtics foresees applications in cancer, but also in auto-immune diseases in which B-cells run wild.
Maurits is convinced that the immunoproteosome is an excellent target. "Whether our current lead molecule can jump through all hoops of the drug development process is less certain, but we can go back to the drawing board to adjust the molecule, we've more options."
Herben cites Maurits' eagerness and the intriguing science as iProtics positives. "Hermen's involvement is also a large asset; he has a good track record." Although winning the challenge is no guarantee for success, it opens doors, says Maurits. "We received a lot of invitations from investors to present our business." Herben: "Investors look at the Challenge as a kind of 'screening', do you get through it, then you are probably worth some attention." ■

iProtics

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“I immediately felt part of a large community”

Tenure tracks

ICI funded four tenure tracks, providing young scientists Kim Bonger, Joost Snijder, Annemarthe van der Veen and Martijn Verdoes the opportunity to start their own research group. They have warm words for the freedom and opportunity they got.

If Martijn Verdoes could have given himself a tip at the start of his ICI tenure track in 2016, he would have told himself to take an extra course in management. Verdoes: “As a principal investigator, you are suddenly not only a scientist, but also a team leader, a financial controller, communications specialist, etcetera. Stuff you’re not trained for. I ‘ve had some very good examples in my mentors, but not everyone thrives on the same way of supervision.” ICI tenure tracker Annemarthe van der Veen (Leidenumc) would have tipped her younger self also that it takes time to build up a team and get results. “As a starting PI, you must be focused and patient. Rome wasn’t built in a day.”

Proud

For a long time, Bonger never imagined herself becoming a professor. She never had examples of female professors with a young family. Now that she’s heading in that direction, she says it’s a reason to persevere. “It is a very nice job, but at times I almost gave up. For example, when I ended up in the top ten percent for an ERC grant but didn’t get it. The tenure track came at exactly the right time.” Recently, Bonger moved from Nijmegen to Leiden, as did Verdoes (also Bongers’ partner). A ‘bittersweet’ decision, according to Verdoes.
“We are very grateful for twelve good years in Nijmegen. In Leiden, I can broaden my focus from tumor immunology to immunology in general.”
Van der Veen has received several other grants since her ICI tenure track started in 2019. “That has allowed me to focus on the research. The coming year, we will publish a lot, and that also gives me a good position to apply for new grants.” Fourth ICI tenure tracker Joost Snijder (Utrecht University): “To prepare for my last meeting with the ICI executive board this year, I read back the plan I made at the start. Not all went as smooth or fast as I would have liked, but reading back made me quite proud of where we stand today. Moreover, with the ERC starting grant I’ve received this summer, I can continue my work.”

Tips and tricks

Van der Veen had just spent fourteen years abroad when she started her tenure track. “Through ICI, I immediately felt part of a large community which helped me and also my PhD-students, a lot.” Bonger: “For a chemist, the immunology field can be quite a puzzle. As tenure trackers, we were involved in organizing conferences, this bulletin, courses for PhDs, etcetera. This helped to connect with immunologists, making the lines of communication very short and establishing collaborations. I quickly knew where to go with a particular question or problem.”
“Of course, I wrote a solid plan with many ideas on what my group was going to do and achieve, but I wasn’t pinned down to the letter,” emphasizes Snijder. “That’s quite unique and a great relief for a starting PI. Science is about improvising and pursuing opportunities.” Verdoes: “I’ve never experienced the yearly progress meetings as a kind of exam or interference. It felt more like coaching and reflection. I received a lot of tips, tricks, and enthusiasm.”

Time is precious

Although all tenure trackers are happy with their current positions, they agree that a tenure track is also demanding and at times stressful. Snijder: “The biggest pressure is the responsibility towards the people in your group, and it would be nice if a day had twice the hours, especially in combination with a young family.” Bonger: “I learned to accept that you can’t do all your tasks perfectly, in the time you have.” She adopted her version of the 80:20 rule: eighty percent of the work can be done in twenty percent of the time. The

Annemarthe van der Veen

Tenure track Leidenumc 2019-2024

“We study how the innate immune system recognizes viruses and what goes wrong in inherited autoinflammatory diseases such as Aicardi-Goutières syndrome (AGS). In such diseases, the body’s own nucleic acids are accidentally recognized by receptors of the innate immune system that normally detect nucleic acids of viral origin. A main goal at the start of my tenure track was to identify some key players in the pathways that control nucleic acid sensing and immunity. We created a relevant disease model and discovered a receptor that is normally involved in viral recognition, but also elicits an autoinflammatory response. This is of clinical relevance, and we are currently working on a strategy to target this receptor and interfere with autoinflammation in AGS or related autoinflammatory diseases.”

remaining twenty percent takes eighty percent. “You need to let go of some of those twenty percent, but that is not easy for perfectionists.”
The tenure trackers eagerly and enthusiastically share their scientific results, but which ideas ended up in the bin? Snijder: “I seldomly give up on an idea, I put it on the slow burner until the right moment comes along.” Van der Veen: “Building up bioinformatic analysis pipelines and support brought some headache, but that’s also running now.” Verdoes is convinced that perseverance wins in science. “If it was simple, it would have been done already.” ■

Joost Snijder

Tenure track Utrecht University 2019-2024

“During my postdoc time, I learned about a major, but not uncommon, problem in immunology: having a highly effective monoclonal antibody but no means to reproduce it. This happens most often after a ‘freezer drama’: your cell line is gone, and the antibody sequence was unknown. There are commercial companies who may save you, but at considerable costs. That puzzle – how to reverse engineer an antibody using mass spectrometry – intrigued me. It was one of my main goals at the start of my tenure track. Although reverse engineering is still no routine job, the software tools and proteomics protocols that we have developed, make it possible, even if only tiny amounts of the antibody are left. Most excitingly, we are now using these tools to understand the human antibody response against viruses. Our papers on the topic are well read, cited, and used. I’m very happy that we could make this method available.”

Kim Bonger

Tenure track Radboud University Nijmegen 2019-2024

“I really enjoy developing new methods for imaging, target discovery and precision medicine. We are also interested in developing molecular probes to understand and modulate cellular mechanisms behind (auto)immune diseases. Our THRONCAT project for example, an improvement of BONCAT - bioorthogonal non-canonical amino acid tagging- has delivered such a nice and successful method. THRONCAT allows the visualization, identification, and quantification of newly synthesized proteins in a cell in response to stress, therapy, or another stimulus. An important benefit compared to BONCAT is that the method does not require a special growth medium. When you add a special threonine analogue and apply your stimulus, the analogue will be built into all newly synthesized proteins and enables visualization by conjunction to fluorescent dyes or affinity tags. The threonine analogue we use is called β-ethynylserine (βES) and was quite unknown. THRONCAT has received much attention and is becoming a new standard, also within ICI.”

Martijn Verdoes

Tenure track Radboudumc 2016-2019

“One of the more straightforward looking projects when I started my tenure track was the creation of a ‘triple A’ conjugate, a tumor vaccine consisting of an Antigen, Antibody, and Adjuvant - all combined in one, single molecule. Promising ‘double A’s’ had already been published: antigen-antibody combinations to guide the vaccine to the right immune cells, and of antigen-adjuvant combinations to increase the immune response. A ‘triple A’ seemed logical and attainable. It turned out to be a much tougher synthetic problem than we foresaw, but we persevered and hope to publish it soon, now. The concept works very well *in vitro*, and *in vivo*, but the pharmacokinetics still needs some work. But we are currently working towards what you may call an AAA^{+} conjugate. We developed a ubiquitin conjugation strategy to solve coupling issues which improves solubility, too. Opening up a totally new line of research in my lab.”

Modified vancomycin promises to become a new antibiotic against MRSA

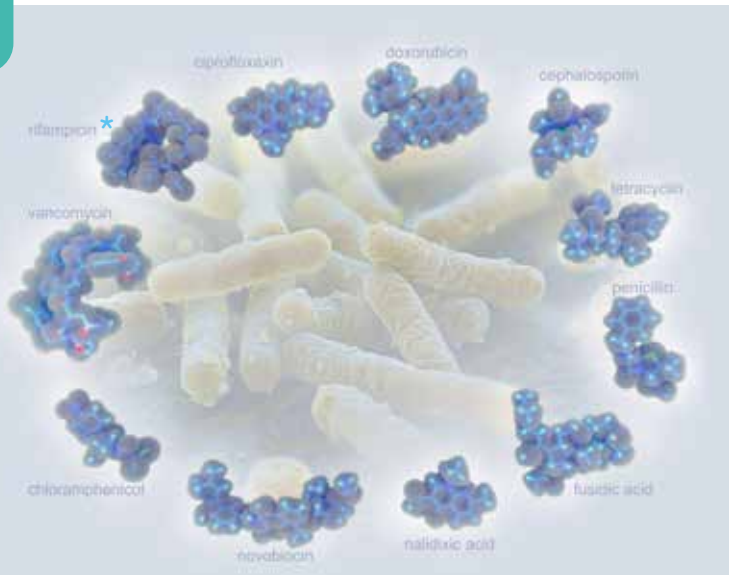
A chemically modified version of the antibiotic vancomycin is much more effective against MRSA and other Gram-positive bacteria than the original. Leiden university professor Nathaniel Martin and his group developed this guanidine modified lipoglycopeptide. They recently published their findings in the journal Science Translational Medicine.

For decades, the methicillin-resistant bacteria *Staphylococcus aureus* (MRSA) could be successfully treated with vancomycin. As a glycopeptide, vancomycin belongs to a different class of antibiotic than methicillin. However, there are issues with vancomycin as well, including side effects caused by high doses. Without appropriate treatment, MRSA infections can be lethal and are estimated to cause more than 100,000 deaths per year, according to a study in The Lancet. Of growing concern is the finding that some MRSA strains as well as other Gram-positive bacteria have also developed resistance against vancomycin.

Efforts to develop alternatives include modifying vancomycin in a number of ways, for example with a lipid tail (lipoglycopeptides), but the resulting antibiotics were often toxic or suffer from poor solubility which is a problem for an injectable. Nathaniel Martin at the Institute of Biology of Leiden University had the bright idea to introduce a guanidine unit with a lipid tail onto the vancomycin core.

"These guanidino lipoglycopeptides have two advantages over vancomycin itself," explains Elma Mons who has been involved in the study in Martin's group for the last three years. "The guanidine moiety carries a positive charge in the body. This causes the antibiotic to bind much better to the negatively charged bacterial membrane and improves its solubility in water. The lipid tail also improves anchoring of the antibiotic in the bacterial membrane. These factors together enhance the antibiotic's binding to lipid II, a precursor of the bacterial cell wall. Binding to lipid II inhibits cell wall biosynthesis, effectively killing the bacteria."

▼ *Molecular structures of common antibiotics: clockwise from rifampicin*, ciprofloxaxin, doxorubicin, cephalosporin, tetracyclin, penicillin, fusidic acid, nalidixic acid, novobiocin, chloramphenicol, vancomycin.*



Well behaved compound

A variant with a tail containing seven carbon atoms, called EVG7 (after Emma van Groesen, the former PhD candidate who designed the original panel of compounds) proved to be the most promising candidate. Numerous studies on the effectivity and toxicity of EVG7 have been positive, describes Mons. "The compound behaves better than any other I've worked with." EVG7 was proven effective against bacteria at a 100- to 1000-fold lower dose than vancomycin. An *in vivo* sepsis study showed that after seven days all mice survived an infection with MRSA when treated with EVG7. In contrast, among the group treated with the same dose of vancomycin, all but one of the mice died. In cell-based toxicity studies, EVG7 showed no cytotoxicity or mutagenicity. *In vivo* renal toxicity studies further showed that at a therapeutically relevant dose, EVG7 caused no adverse effect on the kidneys in contrast to vancomycin.

> ***"One after the other study at effectivity and toxicity came back positive"***

On the way to a new antibiotic

Mons says: "An important next step are biodistribution studies, in which we investigate where the compound ends up in the body after injection. This is exciting but also critical: the antibiotic needs to get to the affected organs, for example skin or other organs in case of an infection after surgery." In addition, the production of the compound needs to be scaled up for larger studies, which Mons is working on. A patent was recently granted on the compound and the method of its synthesis. The researchers are now investigating the possibility for a start-up company which will take on the clinical studies. In a best-case scenario, EVG7 could be a new antibiotic on the market in about ten years. ■

Reference

E. van Groesen *et al.* ***Semisynthetic guanidino lipoglycopeptides with potent in vitro and in vivo antibacterial activity***, Science Translational Medicine 7, Aug 2024, Vol 16, Issue 759.

Developing targeted molecular cathepsin traps to treat cancer

A promising target for cancer therapeutics is the protease family of cathepsins. Researchers from the Swiss EPFL institute in collaboration with Dutch researchers from Radboudumc in Nijmegen succeeded in developing a highly specific, targeted way of inhibiting cathepsin activity. The results were published in May 2024 in the journal Nature Chemical Biology.

Cathepsins play a role in all stages of cancer varying widely from immune cell regulation to matrix degradation. Inhibiting cathepsins with small molecules is a promising target for cancer treatment, but cathepsins are not only functional in tumor cells but also in virtually all normal cells. Specific functions of each cathepsin are becoming increasingly evident. Cathepsin B and L for example are present in virtually all cells, while S and K are mainly found in antigen presenting cells and osteoclasts, respectively.

Inhibiting systemic cathepsin activity can therefore be harmful in many ways. Targeting cathepsin activity must be done more selectively, which EPFL researchers in Lausanne (Switzerland) with help from the Chemical Immunology Lab of Martijn Verdoes at Radboudumc in Nijmegen set out to achieve.

The Swiss researchers led by Elisa Oricchio and Bruno Correia at EPFL first developed a platform to rapidly screen candidate peptide inhibitors (non-natural peptide inhibitors, NNPI). This resulted in some very potent and specific cathepsin inhibitors. "The result is remarkable," says Verdoes. "The approach has produced a new class of inhibitors which are very selective and the binding mechanism has been proven by modeling and crystal structure analysis as well."

Targeted inhibition

The next step was testing the most potent inhibitors on a biologically relevant system. Verdoes is a specialist in developing tools for this, he explains: "In a biological environment many cathepsins are present at the same time. We developed a tool to investigate the biological activity of the inhibitors in a cell or organism, a so-called quenched activity-based probe (qABP)." These probes are reporters of cathepsin activity. The probe is fluorescent only after reacting with a cathepsin. Indeed, in all of the studied cases, adding an inhibitor prevented the binding of the probe to the specific cathepsin and the fluorescence disappeared.

Then, the researchers developed a way to bring the inhibitor to the targeted tumor. They created an antibody-peptide inhibitor conjugate (APIC) which effectively traps a specific cathepsin in a specific cell type. The non-targeted inhibitor is hardly active, reducing the risk of side-effects.

To prove the efficacy of the APIC, a lymphoma was chosen as the target using an anti-CD79 antibody and the cathepsin S inhibitor NNPI-C10. Verdoes explains: "This type of lymphoma needs cathepsin to grow and proliferate, but it also uses cathepsin to enhance the activity of helper T-cells that stimulate growth of the tumor cell. In addition, inhibiting cathepsin activity stimulates cytotoxic T-cells." The researchers showed that the approach works for other tumor cells such as breast cancer cells and osteoclasts as well, proving the wide applicability.

> *"The approach has produced a new class of cathepsin inhibitors which are very selective"*

On the way to clinical trials

Finally, this targeted delivery of potent cathepsin inhibitors was tested *in vivo* in mice. The results are very promising, says Verdoes: "The inhibitors were shown to be very active and were delivered exactly where they need to be. In the case of lymphoma not only the therapeutic molecule was identified but also the mechanism. That is very important for the translation to cancer treatment. The next step now is additional *in vivo* studies and then phase 1 clinical trials." ■

References

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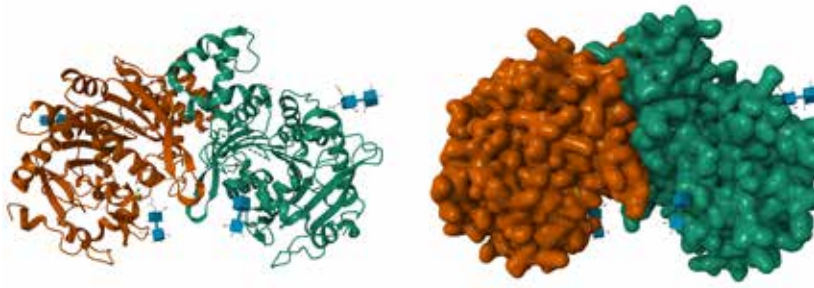
▼ *Cathepsins are proteases found in all animals as well as other organisms. There are approximately a dozen members of this family, which are distinguished by their structure, catalytic mechanism, and which proteins they cleave (photo: crystal structure of cathepsin A, apo-structure).*



FLOW *Protein Quality for Heath*

Mapping the fate of proteins from cradle to grave

In the 2024 round of NWO Gravitation grants, the new consortium FLOW was awarded 23 million euros. Running from 2024 to 2034, FLOW will investigate the cellular factors that determine the fate of proteins throughout their life and learn how to influence that fate.

Correct folding and maintenance of proteins through an extensive quality control (QC) system in cells is essential to our health. Failures can lead to disease, including Alzheimer, Parkinson, metabolic diseases, and cystic fibrosis.
The cellular QC system is managed by several dozens of chaperones, complemented by a wealth of other factors including those catalyzing post-translational modifications and degradation. Together this interactome determines the path of 20,000 proteins through the cell, from cradle to grave. Despite extensive research, there are many gaps in our knowledge, preventing the development of effective therapies.
The coordinator and one of the principal investigators (PI) of FLOW is Ineke Braakman, professor of cellular protein chemistry at Utrecht University. The consortium consists of an extensive team of thirteen other PIs from universities and UMCs all over the Netherlands. “The call that we got the grant came as a big and pleasant surprise. We are elated as we really believe in our program,” said Ineke Braakman.

Mapping protein fate

The path of proteins through the cell can be compared to a subway network. Already during its synthesis, each protein travels from station to station. At each station, a molecular network including chaperones conducts a QC triage to not only help the traveling protein but also decide where the protein will go next. Many of the individual stations and tracks are known, but remarkably, there is no protein for which the entire lifecycle has been mapped.
The aim of the program is to fill in the blanks and understand the entire interactome for two model proteins: Cystic Fibrosis Transmembrane conductance Regulator (CFTR) and alpha-synuclein (aSyn). CFTR is defective in the loss-of-function folding disease cystic fibrosis. Misfolding of aSyn leads to Parkinson’s disease. Together, CFTR and aSyn face every possible fate a protein can experience in cells, making them the optimal proteins to study in FLOW.
Braakman, an expert on membrane-protein folding and of CFTR folding in particular, explains: “Many publications feature CFTR but we still do not fully understand how CFTR navigates the cell and how chaperones and other cellular factors influence CFTR. Even for simple proteins there is no map of their fate from cradle to grave.”

> ***“It tells me that all lights are green for FLOW”***

The reason is that researchers have been using different approaches, says Braakman: “Each protein and each cellular process requires a dedicated assay, which is not necessarily suitable for other proteins or processes. In our biochemical folding studies in cells, large proteins are optimal, because

they undergo many modifications and conformational changes. But for *in vitro* research on protein refolding, smaller proteins are easier and more informative to work with. Every assay has its own ideal proteins. Within FLOW, we aim to develop the approaches to study all life phases for these two proteins, with the intent to expand to many more. We expect that the approaches we will develop will enable us to map the life cycle of other proteins much faster."

From discovery to control

Three work packages constitute the FLOW program: discover, rebuild, and control. The first one aims to determine all possible fates for the two model proteins, produce an inventory of their interaction partners with spatial-temporal resolution, and establish the effects of client and QC-factor variation on the cell.

Next, 'building is understanding' as chemists say: the team plans to recreate the triage processes in the cell that decide on the fate of the two proteins. Braakman explains: "The idea is that when we know how to reconstitute the process, we will also know how to influence it. If, for example, we understand how aSyn misfolds in Parkinson, recreating the misfolding process may teach us how to prevent it."

The last work package aims to reach full control over the fate of these two proteins in cells and expand to tissues and organisms as well as to additional proteins. The ultimate goal of FLOW is to control cell health, and through this, human health.

First results

Many disciplines will collaborate in FLOW, including biochemists, biophysicists, cell biologists, and computer scientists. In a joint hiring effort this summer, the majority of nineteen vacancies was filled with young researchers. The program has its first kick-off meeting in November, which will include speed dates between new researchers of all disciplines. Braakman: "The recruitment symposium already generated a great culture of togetherness among the PIs and the new hires. This collaboration is so important for our research. The questions we aim to answer in FLOW are crucial for cell health and require many disciplines to be answered."

> ***"Remarkably, there is no protein for which the entire lifecycle has been mapped"***

Even in this short time, there are already some interesting achievements, says Braakman. "We recently identified an unexpected motif in CFTR that is essential for its folding process. A new postdoc has already improved an assay for kinetic analysis. I am very happy to see these results so quickly. It tells me that all lights are green for FLOW."

The grant is awarded for ten years, with the second half granted after evaluation. Braakman says: "In five years, we should have the contours of the path from cradle to grave ready for both proteins. We should know what the important interactors are and whether they are unique or redundant. In ten years, we aspire to be able to push a button and change the fate of a protein, for example by inhibiting a specific co-chaperone." ■

FLOW
Protein Quality for Health

Ubiquitin's role in the cell's quality control system

Monique Mulder is one of the principal investigators in the FLOW consortium. Her group at the Chemical Biology and Drug Discovery lab at LUMC specializes in understanding and modulating the role of the ubiquitin proteasome system in diseases, such as Huntington, a neurodegenerative disease caused by protein aggregation. "The ubiquitin proteasome system in the cell is one of the quality control systems. It controls the fate and degradation of proteins by ubiquitination. It therefore fits within the scope of the FLOW program perfectly," Mulder explains.

Mulder and her group use chemistry to understand ubiquitination processes by developing for example activity-based probes, assay reagents and small molecules. So far, they have focused on the ubiquitin enzymes involved in Huntington's disease, but within the FLOW consortium Mulder happily takes on the challenge to switch to alpha-synuclein (aSyn). "It is good to have a focus on the model proteins. The project interfaces with our research, but the protein is new to me. FLOW is a very interesting program as it brings together basic scientists from diverse fields. Many of the PI's are specialized in chaperones, a different but related part of the QC system. It is exciting that we will be challenged to think and work outside our own box."

The first PhD student started on November 1st. First, we will focus on synthesizing aSyn using peptide chemistry. This will allow us to do post-translational modifications (PTM) of aSyn, including ubiquitination. "Many PTMs are known for aSyn, but we don't have a good understanding of their function. We will investigate the interactions and study which of more than 600 known E3 ligases are relevant for aSyn. We will also develop PROTACS which play a role in degradation of unwanted proteins. The overall goal is to be able to prevent aggregation."

What happens when the innate immune system derails in the first step

Project: Innate immune activation and sterile inflammation in microglia in human brain

Timo Oosenbrug is fascinated by immune receptors. "It is the first line of defense against viruses," he explains. "But what makes it even more interesting is what happens when these receptors keep firing all the time, causing sterile inflammation."

As a molecular scientist, Timo Oosenbrug studied the innate immune system during his PhD training in Leiden. Given his fascination, it is not surprising that he jumped at the chance to continue studying immune receptors as a postdoc in the immunology group of Annemarthe van der Veen at the LUMC, among others with an ICI grant.
The innate immune system fights infections by viruses through immune receptors that recognize viral molecules. Van der Veen's group focuses on immune receptors present in the cytosol. These are activated through the presence of viral double stranded RNA inside an infected cell which triggers the production of type I interferons, the onset of an antiviral immune response.
In some people, however, their own RNA is accidentally detected because of a genetic mutation. This triggers sterile inflammation in absence of an infection which causes severe diseases, known as Type I interferonopathies. These almost always affect the brain, such as microcephaly, intracranial calcification and learning disabilities. Patients seldom live long.

Neurons-on-a-dish

Van der Veen's group had already developed cell lines to study the influence of various genetic mutations on the incorrect immune response. The ICI proposal is to study actual brain cells, explains Timo. "Initially we planned to study microglia, the macrophages of the brain, but we shifted to neurons after a major publication showing that neurons may produce double stranded RNA, misleading the innate immune receptors."
In cooperation with the human genetics group of Willeke van Roon-Mom at LUMC, the researchers set out to develop a neurons-on-a-dish model from progenitor cells (descendants of stem cells). "I'm proud that we accomplished the differentiation to neurons. Although neurons are very sensitive cells, we found ways to manipulate them to model various patient phenotypes."

"Cooperation between biology, immunology and chemistry will only intensify in the future"

In a separate research line Timo studied ubiquitination, a post-translational modification that can activate proteins. He found that the ubiquitinating protein HUWE1 plays an important role in sustaining sterile inflammation. "These results are important to understand how the antiviral response works in healthy people, but also to understand how we can inhibit the process of type I interferon production in patients with sterile inflammation."

New biological tool

Combining the research, Timo now plans to study the role of HUWE1 in the newly developed human neuronal cell model. "It is a new biological tool which can be used to study the many factors that play a role in RNA sensing and sterile inflammation. It is an important step in the translation from a cell line in the lab to the patient. Ultimately, we want to be able to identify how we can inhibit the process of sterile inflammation, which could lead to therapy. We have some interesting ideas on this that we are exploring further in the lab."
Even though being a postdoctoral researcher, he has not followed any PhD training programs within ICI, Timo praises the open and inspiring atmosphere within the institute. "At the conferences, people are genuinely interested and helpful. I've attended the meetings with great pleasure." He expects that the cooperation between biology, immunology and chemistry will only intensify in the future. "During my PhD project, immunologists and chemists worked together even before ICI had started. It was a natural way of working." ■

Timo Oosenbrug
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Molecular biology of innate immunity
Annemarthe van der Veen group

NWO grant for RNA research

Last June Annemarthe van der Veen has been awarded an NWO grant (ENW-M1) for her research in the field of vault RNA. All cells in our body contain so-called vault RNAs, small RNA molecules that do not code for a protein. The amount of vault RNA in a cell increases rapidly when this cell is infected by a virus. However, it is not known how large amounts of vault RNAs influence the course of the virus infection. Van der Veen's research group will therefore study the role of vault RNAs during viral infections. The ultimate goal is to discover whether vault RNAs can form a new target for the development of new antiviral agents.

ERC Advanced Grant awarded to Albert Heck

Last April Albert Heck (Utrecht University) has been awarded a prestigious ERC Advanced Grant. The 2.5 million Euro grant enables Heck's team to develop cutting-edge techniques to gain a fundamentally deeper understanding of our immune system. His team focuses on developing mass spectrometry and proteomics techniques to delve into the molecular intricacies of human antibodies.

Forgotten anti-cancer drug for leukemia rediscovered

For years, Sjaak Neefjes and his colleagues have been investigating 'forgotten' chemotherapy drugs for effective cancer treatment. In their recent publication in Molecular Cancer (June/2024) researchers from both the Leiden University Medical Center (LUMC) and the Netherlands Cancer Institute share their remarkable findings on the drug aclarubicin – an effective blood cancer drug used in China and Japan, but not (anymore) in the Netherlands.

Recent publications

Schwerdtfeger F et al

The conformation of tetraspanins CD53 and CD81 differentially affects their nanoscale organization and interaction et al with their partners

Journal of Biological Chemistry, Volume 300, Issue 9, 2024. DOI:10.1016/j.jbc.2024.107685

Qiao X et al

Diversifying the anthracycline class of anti-cancer drugs identifies aclarubicin for superior survival of acute myeloid leukemia patients

Mol Cancer. 2024 Jun 4;23(1):120. DOI: 10.1186/s12943-024-02034-7. PMID: 38831402

Petruzzella A et al

Antibody-peptide conjugates deliver covalent inhibitors blocking oncogenic cathepsins

Nat Chem Biol. 2024 Sep;20(9):1188-1198. DOI: 10.1038/s41589-024-01627-z. Epub 2024 May 29. PMID: 38811854

Roet JEG et al

Unbiased method for spectral analysis of cells with great diversity of autofluorescence spectra

Cytometry A. 2024 Aug;105(8):595-606. DOI: 10.1002/cyto.a.24856. Epub 2024 Jun 12. PMID: 38863410

Venrooij KR et al

Mutually Orthogonal Bioorthogonal Reactions: Selective Chemistries for Labeling Multiple Biomolecules Simultaneously

Top Curr Chem (Cham). 2024 Jul 6;382(3):24. DOI: 10.1007/s41061-024-00467-8.
PMID: 38971884

Zyla DS et al

A neutralizing antibody prevents postfusion transition of measles virus fusion protein

Science, 28 Jun 2024, Vol 384, Issue 6703. DOI: 10.1126/science.adm8693

Schulte D and Snijder J

A handle on mass coincidence errors in de novo sequencing of antibodies by bottom-up proteomics

Journal of Proteome Research, June 27, 2024, Vol 23/Issue 8. DOI:10.1021/acs.jproteome.4c00188.

Weiss L et al

Immunofilaments are well tolerated after local or systemic administration in mice

ACS Pharmacol Transl Sci. 2024 May 3;7(6):1874-1883. DOI: 10.1021/acsptsci.4c00180. eCollection 2024 Jun 14. PMID: 38898947

Column

THE GAP BETWEEN IMMUNOLOGY AND CHEMISTRY IS CLOSED – OR IS IT?

HERMEN OVERKLEEFT
ICI EXECUTIVE BOARD

Professor of Bio-organic Synthesis at Leiden University

When the Institute of Chemical Immunology was established, this was met with enthusiasm and scepticism. Enthusiasm for the large pot of money. Scepticism, because immunologists considered chemists useful only for the creation of tool compounds. Chemists viewed immunologists as FACS afficionados who dreamed of identifying yet another T cell. Open-minded researchers from both disciplines joined forces to overcome these biases.

ICI was established by experts in innate and adaptive immunity, by expert cell biologists and animal scientists. These were matched by experts in bioorganic chemistry, in structural chemistry and in analytical chemistry. A large and loosely defined area of immune-related diseases including cancer and autoimmune diseases was chosen and twinning projects defined in which chemistry and immunology PhD students worked together in interdisciplinary projects. The call for new Tenure Track staff yielded four now established researchers, some of whom endow themselves with the title of Chemical Immunologist. Senior and junior researchers identified common ground for their current research, with many exciting findings as a result. They learned each other's language. The field was established.

ICI will however cease to exist. Attempts to acquire funds to create ICI 2.0 have failed. Failed, because both sides seem to have retreated behind the walls of their monodiscipline. Failed also because of wavering leadership, with the next generation looking at the original ICI leadership for guidance, who wished to pass on the baton. Chemical Immunology is now in an existential crisis. How to make Chemical Immunology a lasting discipline? The solution is simple.

We need to keep on meeting. To keep discussing progress in our respective disciplines. To keep on pushing new chemical methodologies, and also to voice new immunological challenges. To identify and address new and exciting interdisciplinary research questions and find funds to address these. But this requires an effort. Are we willing to do so when participation in meetings is on a self-funded basis? Are we able to further each other's science when no funds are directly available? Only if we are, chemical immunology will become a mature and lasting field. ■

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 email to info@chemicalimmunology.nl.

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