WHY WE STUDY VIRUSES

April 30, 2018

Farewell Symposium professor Alexander Gorbalenya

VENUE: STADSGEHOORZAAL

09.45 **Welcoming address: How viruses affect our lives**
Louis Kroes | Department of Medical Microbiology, Leiden University Medical Center

CHAIR: ELLIE EHRENFELD

10.00 **The love triangle in RNA viruses: replication infidelity, genome stability, evolvability**
Vadim Agol | Institute of Poliomyelitis and Viral Encephalitides, Chumakov Scientific Center for Research and Development and Lomonosov Moscow State University, Russia

10.35 **Evolution of the virus world: a 30-year journey**
Eugene Koonin | National Center for Biotechnology Information, NIH, Bethesda, USA

11.10 Coffee

CHAIR: IGOR SIDOROV

11.30 **Why I started and continued to study virus evolution and diversity**
Chris Lauber | Institute for Medical Informatics and Biometry (IMB), Carl Gustav Carus Faculty of Medicine, Technische Universität Dresden, Germany

12.05 **Of spots and spines; a trip into the polyomavirus**
Mariet Feltkamp | Department of Medical Microbiology, Leiden University Medical Center

12.40 Lunch

CHAIR: MARJOLEIN KIKKERT

13.50 **Coronavirus phosphodiesterases that antagonize innate immunity**
Susan Weiss | Perelman School of Medicine, University of Pennsylvania, Philadelphia, USA

14.25 **Nidovirus enzymes: a 30-year expedition into uncharted territory**
Eric Snijder | Department of Medical Microbiology, Leiden University Medical Center

15.00 *Transfer to Academy building*

VENUE: ACADEMY BUILDING

16.15 Why I study viruses
Farewell lecture professor Alexander Gorbalenya

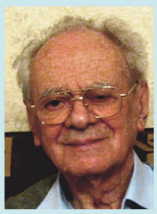
17:00 Closing remarks

17.30 Reception

WHY WE STUDY VIRUSES

speakers

LOUIS KROES graduated in Medicine at the University of Amsterdam, did a PhD study at the Erasmus University Rotterdam in biochemistry and hematology and was trained as a clinical microbiologist. He currently is professor of medical microbiology, in particular clinical virology as well as head of the Department of Medical Microbiology at LUMC. Fields of interest include viral infections in the immunocompromised host (cytomegalovirus, Epstein-Barrvirus, adenovirus), antiviral therapy and congenital viral infections, including parvovirus B19.



VADIM AGOL graduated from the 1st Moscow Medical Institute (1951) and joined the Institute of Poliomyelitis and Viral Encephalitides (1956), where he is working until today. He participated in the organization of the Department of Virology of the Moscow State University (1963) and founded the Department of Virus-Cell Interactions (1965) at the Institute of Physical-Chemical Biology of the same University. His research is focused on the molecular and cellular biology, evolution, and pathogenicity of RNA viruses.

EUGENE KOONIN is leader of the Evolutionary Genomics Group at the National Center for Biotechnology Information and member of the National Academy of Sciences of the USA. He received his Ph.D. in molecular biology in 1983 from Moscow State University, joined the NCBI in 1991 and became a senior investigator in 1996. His research interests focus on evolutionary genomics of prokaryotes, eukaryotes and viruses, and on the general theory of the evolution of life.

CHRIS LAUBER graduated in bioinformatics at the University of Jena and performed his PhD research in the Gorbalenya group at LUMC where he studied RNA virus evolution. Currently, he is a postdoctoral researcher at the University of Technology Dresden. His work is at the intersection of computational biology and virology and he is particularly interested in virus discovery, macroevolution of viral genetic diversity and the interplay of viruses and their hosts at the systems level.

MARIET FELTKAMP is consultant and associate professor in medical virology at the LUMC. She studies clinical, epidemiological and molecular aspects of human papillomavirus (HPV) and polyomavirus infection. Her lab discovered the trichodysplasia spinulosa polyomavirus in 2010 and received the 2018 Novartis Transplantation Award for predicting BK-polyomavirus infection in kidney transplant recipents. She is member of the ESCV executive board, the ICTV Polyomavirus Study Group and the HPV committee of the Dutch Health Council.

SUSAN WEISS obtained her PhD in microbiology from Harvard University and did her postdoctoral training in retroviruses at the University of California, San Francisco. She is currently professor of microbiology and associate dean for postdoctoral training at the University of Pennsylvania. Her long-term research interest is in coronavirus pathogenesis, with current focus on virus interactions with the host innate immune response. Additional recent interests include Zika virus-host interactions and pathogenic effects of host endogenous double-strandedRNA.

ERIC SNIJDER graduated from Utrecht University (MSc in molecular biology and PhD in virology) and moved to Leiden University in 1990. He currently is a professor of molecular virology and head of the section Research at the LUMC Department of Medical Microbiology. His long-term research interests are the molecular biology and evolution of (emerging) +RNA viruses, in particular nidoviruses, their multifaceted interactions with the host, and the development of antiviral strategies.

organizers

Why I study viruses

Alexander E. Gorbalenya

Hooggeleerde collegae, zeer gewaardeerde toehoorders. Ladies and gentlemen, friends, and family.

The Beginning: Viruses are Pathogens

Among the fine people I was fortunate to meet during my student years in Novosibirsk, there were two, Vladimir Gruntenko, the supervisor of my Master Science project, and Sergey Tychkov, a friend of mine, who died prematurely from neurodegenerative diseases. They were wonderful people and creative scientists with many ideas left unrealized. At least in one of these two cases, a virus hit-and-run incident was suspected.

Although severity and type of disease may vary with virus infections, they are very common from the moment we are conceived. This association with disease makes virus infections part of our collective human experience. It is through causing disease that viruses were first independently identified by Dmitri Ivanovsky in Russia, Martinus Beijerinck in The Netherlands, and Friedrich Loeffler and Paul Frosch in Germany, 120 years ago. I would like to believe that it is not a coincidence that, at this moment, when I am reflecting on viruses, most people in the auditorium are connected to one or several countries where virology was born.

Because of their link to diseases, viruses are *pathogens*. This characteristic defines the place of viruses in both the public perception and scientific research, carried out by virologists on behalf of the public. It is what motivates young people to join virology and it defines the ultimate goal of studies in virology: to contain, prevent and cure virus infections. It is what was expected from me when I was hired by the Leiden University Medical Center to do my research at the Department of Medical Microbiology as well as by external funders of my research. And it is what I have tried to accomplish over the years working at the LUMC, and last but not least, it is what I consider a noble cause for the future.

However, when I look back on my 40-plus years in virology, I see that the fight against viral diseases has only been part of the broader motivation of ‘why I study viruses’. My perception of viruses and virus research has been shaped by many factors. It has

evolved along with the advancement of virology and my knowledge of viruses. It has also been affected by my experience in other domains of life. I touched upon some of these aspects in my inaugural lecture at this distinguished place some years ago.

In the next 40 minutes or so, I'll try to share with you my appreciation of different facets of viruses, and I'll describe how they may be interconnected. Additionally, I would like to share with you a few unique moments when I was stunned by wonder and beauty of what I observed or deduced during my research of viruses. These cherished moments were experienced thanks to the generosity of many, some of whom are here today, some of whom who unfortunately cannot attend, and some of whom who are sadly no longer among us. When I think about 'why I study viruses' all these factors come together.

The Beginning: Viruses are Small

I started studying viruses for purely *opportunistic* reasons that included seeking a fresh start and taking on a position that promised access to modern research facilities, career growth and economic stability. At the time, I knew almost nothing about viruses, which were barely present in the natural science curriculum at the Novosibirsk State University where I studied. Before I opted for virology, I spent three years, two undergraduate and one post-master, in a lab studying a complex phenomenon of mice biology at the interface of genetics, immunology, endocrinology and cancer research. My project was interesting and challenging, and I learned without prior medical training to graft organs in new-born mice. However, I felt that the enormous complexity of the project did not match the available resources and techniques, let alone my limited capabilities. In this respect, switching to the study of viruses seemed to have been a sensible alternative. Viruses are exceptionally *small,* so it must be straightforward to study them, I reasoned.

My first virus project was with Konstantin (Kostya) Chumakov in Professor Vadim Agol's lab at the Institute of Poliomyelitis, Moscow region. Kostya, a few months younger than me, but already a seasoned virologist much to learn from, was a pure joy to watch when he was working. Nationally, Vadim Agol's lab was leading in virus research at molecular level, the new frontier at the time. Its top reputation was the reason for my new employer in Novosibirsk, Institute of Molecular Biology, to send me there for one-year training, and it was an honour for me to be admitted. When I

joined the lab in December 1975, I didn't know that it would be the place I'd stay associated with for the next 22 years, while also working at or visiting other places in the country and abroad. While based there, I visited the laboratories of Willy Spaan in Leiden, Michael Rossmann in West Lafayette, and Stuart Siddell in Würzburg for sabbaticals of several months.

The virus I was going to study caused severe brain and heart diseases, and was named accordingly encephalomyocarditis virus, or for brevity's sake, cardiovirus. Only few groups worldwide made it a centrepiece of their research programs. One of these was Agol's lab. This virus was benign for humans which simplified the logistics of the experiments. But there was the primary reason to work with this virus: it was a distant cousin of two viruses of public significance. They were the poliovirus which causes poliomyelitis in humans and the highly contagious foot-and-mouth disease virus of cattle. In the unlikely case you missed it, the poliovirus was for my and earlier generations what the human immunodeficiency virus or HIV became for later generations decades later. Because of its relation to these two major viruses, the results obtained with the mouse cardiovirus was also considered valuable. About three years after I joined the lab and became a PhD student with Vadim, my first paper in virology was published, which reported an unexpected biochemical property of an obscure protein of the cardiovirus. However, it was the second cardiovirus protein I studied that influenced my entire career. I'll return to this protein, after reflecting on what I believe motivates people to do scientific research and exploring a case for beauty in virus research.

How we do science: curiosity as a driving force

When I was working on this lecture, I came across a provocative statement attributed to a nobleman named Tancredi from the novel "Leopard" by Giuseppe Tomasi di Lampedusa, made also into a movie by Luchino Visconti. It reads "*a palace in which one knows every room isn't worth living in*". Taken at face value, this statement may sound as arrogant as anything to which only few privileged or super rich could really relate to. On the other hand and when not taken literally, this statement may resonate to many who make science their *palace*. Let's see how this palace may look.

For the subsequent journey, I'll borrow from a parallel that was introduced by Professor Jelle Goeman, a fine statistician and collaborator of mine, in his recent inaugural lecture

at this distinguished place. He compared the process of gaining knowledge in science with trying to describe the interior of a completely dark room by a researcher who moves around and touches every surface. After a while, and assuming that the researcher is persistent, we may expect that every object in the room will become defined and its position established. Jelle used this parallel to show how the reasoning of decision-making could work in different research fields. I suggest we extend this parallel, and ask what would happen to the researcher after he or she has successfully described that dark room? Surely, the researcher may be lured into the next dark room, and then to another one and so on, until the interiors of every room have been fully mapped. Would the mission then be accomplished? For this fictitious example with the rooms, probably yes, but if a parallel is drawn with exploration in science: not really. It is because the notion of the *last* room does not hold for science. Indeed, the beauty of science is that the true boundaries of Nature remain unknown, and it is the unknown which drives scientists in their research, or, if you wish, their wandering through never-ending rooms. If the opposite had been true, and all rooms could have been counted, many people who are driven by *curiosity* might never have joined scientific research. In this respect, researchers may relate to the nobleman's idea of what makes a palace worth living in, which I have just cited.

To be accurate, there is another way to explore the world, and it may be instructive to describe it as well. If we go back to the parallel between a palace with lots of rooms and Nature, we can imagine a room the walls of which may never be reached and which is packed with an endless number of objects which can move, change shape and the size of which may vary from miniscule to giant. In other words, it is a truly magic room containing a lot of diverse and peculiar objects. Once a researcher gets into this room, which is dark, he or she may spend his or her entire life mapping its interior and never describe more than just a tiny corner. Still, this very limited success – if measured against the scale of what should be accomplished – could bring a lifetime of fulfilment to the researcher. It is because it is measured against what was known about this room when the exploration started. Now imagine a remarkable palace made of these magic rooms that are somehow connected. After entering this palace, a researcher may get lost in a single or in many rooms, while mapping objects in the process and never reaching the walls. That wandering may be a loose approximation of how and why researchers do studies in science. And that also includes a specific reason why I study viruses: *I am just curious*!

Like Formulas and Poems, Viruses are Grand Connectors

Now let me make a case for another reason why to study viruses. Curtis Suttle, a Canadian pioneer of virus discovery in oceans, describes the world of viruses as a universe of stars. Just imagine you go to a remote place free of light pollution on a cloud-free night and observe a sky full of stars. Many people will have experienced that in their lives, and if you are among those lucky, you can link it to viruses. What I find truly remarkable in this parallel is that it also creates, unconsciously, a poetic image of viruses. It is something that is very difficult to accomplish, since this image clashes with the innate pathogenic perception we have of viruses, deep inside us and not particularly positive. That is why I am jealous of astronomers whose topics of research are not subject to a comparable negative public perception. Despite, we shouldn't forget, cataclysms caused by celestial objects coming occasionally from the sky to this planet and affecting the biosphere and human civilizations in an awful way. Simply sharing the beauty of the starry night sky, astronomers can and do attract an army of followers of all ages to their discipline. Some get hooked for life and become professional stargazers.

Apparently, I am not alone in this respect, and the aesthetics of science is something many seem to be fascinated with, and some try to reveal it. Here, on the walls of old and new Leiden houses, you can see famous formulas made known to the public through the work of renowned physicists who walked the streets of this very city. These formulas connect forces, energy and mass in ingenious ways revealing the dynamics, boundaries and infinity of the physical world we live in. The very same, but now for the world of the human soul and body, we find in good poetry written in many different languages. It's no coincidence therefore that these two projects, celebrating formulas and poems, intertwine on the walls of this town in an increasing number of places.

When I see this beautiful fabric I can't help but noting that, like formulas and poems, viruses too are grand *connectors*. They do it uniquely and in many ways, by uniting physical and biological worlds as well as all species of the biosphere, while defining the limits of natural diversity. May we therefore expect virus images to adorn the walls of Leiden in the future?

Well, it may be a bit far-fetched, if only because virology is a much younger discipline than physics, let alone poetry, for people to appreciate it in this lovely way. However, I do hope that the case for beauty in virology does have merit. I'll take the liberty to describe a few moments from my research life when I felt particularly stunned and captivated. That complex experience remained when I thought back of those moments later on: absolute wonder and a sense of beauty, momentous and inseparable.

Producing a Beauty in Virus Research: Birnavirus Protease Dissected

To put yourself in the shoes of a researcher, it is important to realize that in their studies researchers seek to gain insight and collect evidence to prove it. Scale and detail of insight and reliability of its evidence are two chief factors that separate different studies. In biology, it is notoriously difficult to obtain direct (or undisputable) evidence of any insight. Researchers rather combine a lot of indirect evidence gathered with different techniques to arrive at conclusions considered consistent with all observations and thus reliable. Those evidence and conclusions must be accepted by peers and also survive the test of time, which is scrutiny by studies of other researchers, before they can take their final place in the 'tower of science', so to speak. It is for this reason that completing a single-paper study which meets four major criteria – namely, deep and broad insight, multidisciplinary evidence, unconditional peer approval, and independent reproducibility – is rare and amounts to something very *beautiful*. I believe it happened only once in my life which I'll try to share with you in a moment. For my recollection, I will connect terms of science to matters of everyday life in order to retain accuracy and make the story accessible at the same time.

My rare moment of wonder and beauty was realized in a collaborative study with Egbert Mundt, a global authority on poultry vaccine development, who is currently with Boehringer Ingelheim. At the time, Egbert was at the Insel Riems Friedrich-Loeffler Institute in Germany, while I was working in Frederick, MD, USA as a visiting scientist at the Advanced Biomedical Computing Center of the National Cancer Institute. Egbert and I combined our expertise for dissecting the molecular mechanism of the formation of virion particles of an RNA virus, known as avian birnavirus. Virus particles were used to develop a vaccine against this virus, which causes outbreaks of an immunodeficiency disease in birds and presents a continuing threat to the poultry industry. Against this background, our study was a basic research linked to the immediate needs of society.

Joining the collaboration, Egbert had established a state-of-the art technical platform for characterization of the birnavirus infection in tissue culture, where this virus could be propagated, and for engineering mutations into the virus genome. My role was to use comparative genomics and biochemistry to guide experiments concerning identification and characterization of an enzyme, which scientists call *protease* and which was implicated in the formation of virus particles. To convey this story I have to present a brief explanation of the terms which I will use further.

All proteins are biological molecules that are made of 20 types of amino acid residues, sort of bricks, that are connected in linear chains including several hundreds of amino acids on average. When produced, proteins are folded into three-dimensional structures, the shape and other characteristics of which are critical for their function. Some proteins function as enzymes that accelerate chemical reactions inside and outside cells. Enzymes include several amino acid residues responsible for their enzymatic or catalytic activity; these residues are called catalytic. Due to their exceptional role, these residues have special chemical properties and their replacement by other residues in nature or in a laboratory commonly kills the affected enzyme.

In our study, Egbert and I were interested in a certain kind of enzyme, known as proteases. These enzymes promote the cleavage of other proteins into pieces of different sizes. In the case of the studied birnavirus, a protease cleaves a precursor of several proteins, called polyprotein, to release proteins which then form virus particles. Due to their cleavage activity, proteases may be compared to knives, and like knives - which come in many sizes and types in households and elsewhere - proteases do exist in many forms and serve different purposes. For many years before this study, researchers failed to identify a protease which was involved in the formation of virus particles of birnaviruses.

Our study started with the input from my bioinformatics analysis which suggested that the enigmatic protease might be a part of the birnavirus polyprotein. This arrangement - that a protease and its target (or substrate) were part of the same protein - was common in RNA viruses, although it may look a bit strange if applied to knives. It is like imagining a knife being part of a French baguette. Surely, a weird combination as such, but offering the convenience of having a tool at hand to slice this baguette from the moment you buy it. As we know, people do not use a knife to slice a baguette on the spot: they use their bare hands if needed. However, having a knife attached to its target

ensures the cutting is precise and fast, very important considerations when applied to the mass production of millions of birnavirus particles.

Back to the project, my prediction about the protease identity proved to be correct in experiments of Egbert and his PhD student Christoph Birghan. They showed, using engineered mutants of the predicted two catalytic residues, that the polyprotein was no longer cleaved and virus particles were not formed. The effects could be compared to using a knife of which the sharp side of the blade was no longer different from its opposite side: it did not cut bread any longer. Furthermore, we were able to convert the birnavirus protease into another catalytic type with the altered properties, the trick that works only occasionally. It could be compared to converting the knife blade from one type to another, for instance from the bread type to the meat type. The meat knife could still slice the bread but not as fast as the original does. In cells infected with this mutant – never found in nature – we observed the polyprotein was cleaved properly, albeit slowly. That mild defect produced a disproportionally large effect on virus replication that made the mutant virus nonviable, revealing dependencies not known before. We also obtained evidence for the catalytic roles of the two candidate residues that could satisfy biochemists. To do so, we reproduced the polyprotein cleavage outside the virus infection, or *in tube* as scientists would say. That system allowed us to prove selective sensitivity of the birnavirus protease and its mutants to specific changes of the reaction conditions, which could be attributed only to the two candidate catalytic residues working in concert and with a defined chemistry. Those results have put the identification of these residues and the mode of their activity beyond reasonable doubt even for the most demanding experts, an advancement uncommon in virus research. Collectively, the different lines of our research generated a profound, multi-level and reliable insight into birnaviruses that exceeded our wildest expectations.

However, that was not the end of the story. The impact of our study was felt beyond birnaviruses or even virology. We tested and verified the proteolytic activity of the specific birnavirus protein because of its statistically significant sequence similarity with an established protease of bacteria, which was uncovered during my bioinformatics analysis. Based on this similarity, the two proteases and many more poorly characterized proteins from Archaea and mitochondria of eukaryotes could be considered evolutionary related or homologous. In a surprising twist produced by our study, the birnavirus protease became characterized in finer detail than the bacterial

homolog which guided its discovery! As a result, we were in the position to return the "favour" and informed the bacterial research community about the most likely catalytic mechanism of proteolysis of this bacterial protease. Our insight solved a long-standing enigma of the catalysis by this protease, which is central to the wellbeing (or homeostasis, as scientists call it) of bacteria upon stress. (Yes, bacteria can also be put under stress, we don't like it and nor do they!). And in their response to stress bacteria rely on the protease that is related to the birnavirus protease.

Over the years, our results were reproduced and confirmed by others. They validated a notion that birnaviruses, bacteria and other organisms, including humans, use homologous proteases of the rare catalytic type for the very different biological processes that are central to their survival.

Our study, conducted by only three people, was completed and published within two years, which was very fast for the reported scale of the reliable multidisciplinary insight, as many of you can attest. I managed to co-author nothing comparable in another *single* paper in my entire research career. And I was very fortunate to collaborate with many fine investigators. It would take many lectures like this to summarize insights gained in projects with Eric Snijder, Bruno Canard, Susan Weiss, Michael Lai, Kouichi Morita, Jens Herold, Volker Thiel, Stuart Siddell and John Ziebuhr on diverse nidoviruses, or Ellie Ehrenfeld, Natasha Teterina, Denise Egger, the late Kurt Bienz, Frank van Kuppeveld and Eckard Wimmer on picornaviruses and Mariet Feltkamp on polyomaviruses. Susan, Eric and Mariet highlighted some of our findings in their lectures at the Symposium today. And there were many other collaborative studies that may have been equally or even more impactful than the birnavirus protease project, and it was an honour to present their results on other occasions.

Birnavirus protease study: Connecting People, Viruses and Advancements in Time and Space

With the observed rate of one case per 200 or so publications that I have co-authored, the birnavirus protease study was a chance event, using the 1% threshold of the p-value statistic. And, indeed, pure luck – or chance – played its part in the genesis of this beauty, and I feel forever grateful to be its recipient on that occasion. It's good to know that one can be lucky! However, besides luck, there were other very influential factors,

which realized this study and affected my choices and other studies I may not present in such detail. These considerations played a significant part in my decision to tell this story on this extraordinary occasion. In the next ten minutes or so I will place the birnavirus protease project within temporal and spatial networks that include remarkable people, diverse viruses and amazing advancements.

It was the year 2000, the change of the millennium, when Egbert and I published our birnavirus study. I was in the middle of my research career and in transition, moving from Russia via the USA to The Netherlands. It was the late Don Summers, Associate Director at the National Cancer Institute's Research and Development Center at the time, who invited me to Frederick, Maryland, to continue my studies. When I was still in high school, Don together with his long-time collaborator and friend Jake Maizel as well as two other teams, led respectively by David Baltimore and Jim Holland, independently challenged the "one gene, one protein" paradigm of molecular biology. They demonstrated that the poliovirus genome encodes a single polyprotein, which is cleaved into many functional proteins during the poliovirus infection, being equivalent to the "one gene, many proteins" relation. It was a revelation, and it was the very same phenomenon that Egbert and I dissected in the birnavirus study. Neither Don nor I knew that my research would be connected to his discovery when I moved to Frederick. Two years later in 2002 when I just started my studies here at the LUMC on the invitation of Willy Spaan, Jake Maizel and I in collaboration with Michael Lindberg from Kalmar University in Sweden published a paper on a new picornavirus that Michael discovered. It was dedicated to the memory of Don Summers.

In fact, Jake and Don influenced the birnavirus project also in other significant ways. In my PhD study in Vadim Agol's lab, I used the technique of electrophoretic separation of proteins that was pioneered by Jake. Along with a substrate provided by Yuri Svitkin it made possible the identification of the virus protease that cleaved the polyprotein of the mouse cardiovirus, a poliovirus relative, which I described at the beginning of my lecture. In my further biochemical research with Yuri, I assigned this protease to a specific catalytic type, using a "trick" that was reused in the birnavirus protease project 20 years later.

My pursuit of understanding the catalysis by the cardiovirus protease, also had an unexpected implication: It prompted me to use bioinformatics, which affected all my subsequent research, including the birnavirus protease project conducted many years

later. That fateful development happened while I was writing my PhD thesis in Novosibirsk. At the time, Eckard Wimmer and his team published a landmark paper that described the entire genome and polyprotein sequences of poliovirus along with the location of all proteins in the polyprotein. It was the first genome sequence of the RNA virus of eukaryotes, and it radically changed how research into the poliovirus and related viruses was to be conducted from then on.

The genetic code of the poliovirus, or its blueprint as they say, offered the complete information about this virus at genetic level which was exceptionally reliable. It was amenable to computational analysis within diverse statistical frameworks. Due to external and internal constraints on the evolution of viruses, different elements of its code, which are nucleotides of genome and amino acids of proteins, change at different rates upon different degrees of freedom. Remarkably, similar constraints may operate in different viruses and host organisms under certain conditions. When sequences of genomes and proteins of these entities are compared, variations in the rate of change of different code elements may be revealed. Specifically, slowly evolving elements may form patterns that could tell researchers about the function, structure and evolution of the compared entities. This technique is known as comparative sequence analysis and it forms a branch of bioinformatics. Both disciplines were nascent and hardly used in virology at the time when Eckard Wimmer released the poliovirus polyprotein sequence, which I decided to analyse to learn about the cardiovirus protease.

To cut a long story short, that decision proved to be fruitful. It was thanks to two very talented fellows: Vladimir Blinov, physicist by training, and the late Alexey Donchenko, mathematician, who enthusiastically joined in the project. None of us held a PhD, and we worked in our spare time, which was sufficient for this purely theoretical study with only a few protein sequences under analysis. Vladimir had already trained his eyes on sequence pattern recognition, and Alexey developed software for our analysis. With the help from our friends we ran this software on main-frame computers. We mapped the cardiovirus protease and its principal catalytic residue on the poliovirus polyprotein. We discovered a second protease in the poliovirus polyprotein, and revealed homology between the two poliovirus proteases and a class of host proteases that belong to a different catalytic type. Furthermore, our analysis identified striking sequence patterns in the poliovirus polyprotein that suggested a primordial origin of poliovirus from primitive self-replicating molecules.

The obtained results challenged the long-held perceptions of different research communities. At the time, most virologists believed that RNA viruses may have had no sequence similarity to other biological entities and that they must have originated from cells relatively recently. Enzymologists and structural biologists maintained that enzymes of two different catalytic types could not be related. Our results suggested otherwise and I was shaken by the enormity of their implications.

It was also striking to observe the apparent ease and speed of our analysis, compared to what I experienced in the biochemistry lab. Over many years, I had spent countless days and nights in the lab, washing glassware and making solutions to prepare and conduct my experiments, which failed most of the time. When my collaborators and I eventually succeeded and identified the cardiovirus protease using conventional methods, our result was a breakthrough. This protease was only the second one identified in any virus and the first of the most numerous class of viruses, known as positive-stranded RNA viruses, and I felt proud of this discovery. My mentor Vadim Agol and his superiors considered my effort and its result worthy of offering me a permanent job and relocation to Moscow from Novosibirsk, which was an exceptional decision since it involved, among other things, red tape nightmare for Vadim and our colleague Lucy Romanova. However, compared to the results of the comparative sequence analysis of the poliovirus genome my prior accomplishments paled into insignificance.

And my two collaborators on the project felt this difference too. Vladimir Blinov got hooked on comparative sequence analysis of viruses and has stuck with it ever since. Alexey, the mathematician, gradually fell in love with biology and tried a stint at Alexander Blinov's lab at the Institute, where I conducted my Master Science project. He learned what I had learned before in my lab days: It is tough to do experiments. When Alexey passed away prematurely years later, he was with an opera company at the main theatre in Novosibirsk.

The only problem with our comparative sequence analysis was that its bold conclusions, if not the results, might have been a delusion, either real or perceived. That was my chief concern, which I have tried to address in various ways in my bioinformatics projects ever since. That is why we invested in the advancement of software to facilitate comparative sequence analysis and assess its statistical support; why I sought collaboration to verify functional and structural implications of our

comparative sequence analyses, and why I hold the birnavirus protease study in such high esteem as a fine example of the synergy generated in the cross-disciplinary collaboration involving bioinformatics.

Fast forward several years and already in Moscow, I completely switched my research to bioinformatics. That move was against the original plan and made possible thanks to the generous support of the new research direction by Vadim Agol who gave me full freedom in this respect. One of the results was that Eugene Koonin, who was my roommate for years, joined my bioinformatics research. With our Novosibirsk collaborators we improved the software for sequence analysis in such a way that it was also instructive in the birnavirus virus protease study many years later. Eugene's extraordinary qualities allowed us to expand the research considerably and to publish the obtained results to achieve international prominence. Without his contribution I would not be here now. Eugene has continued his research most successfully, presenting selected results at the Symposium today.

The virosphere is immense and we are at the very beginning of its exploration

When I started my career in virology, only a hundred or so different viruses had been identified in the previous 80 years by exploring the virus world, or *virosphere* as scientists call it now. That was 40 years ago. Gradually but increasingly faster due to a technological revolution of recognizing viruses, things changed dramatically. Nowadays, virus discoveries are part of routine reports, and so many are identified that it becomes impossible to say accurately how many different viruses we know at any particular moment.

I'll return to this aspect in a moment after we have inspected the consequences of yet another extremely important development concerning the number of viruses. Virologists realized that the total number of *different* viruses on our planet, or the virosphere size, must be astronomically large. It is because we know no biological species, or hosts as virologists say, which are definitively free from virus infections. So the virus diversity may not be smaller than the host diversity. To get an idea of how large that number of different viruses may be, let's compare a virus to a room, and imagine that our planet with all its different viruses is a palace full of rooms. Now let's imagine that a researcher wants to inspect every virus on this planet in his or her lifetime. This would mean a journey through every room of this marvellous palace. To

be up to this task, the researcher should have to move really fast through the palace, spending just a single second or so in every room, and do this every day around o'clock from the first second of his or her life and for the entire 100+ years. And even with a pretty long and busy life and at this astonishing speed, which may exceed the current rate of virus discovery by a 100 times, the researcher may not visit every room. In this sense, one may never know how many rooms there were in that palace, or - returning to viruses - how many different viruses circulate on this planet.

The above parallel may also be useful to put the fraction of viruses that we already know in a familiar perspective. This would be the same as asking *how long* must a researcher be wandering with the speed of one second per room in the part of the palace containing rooms with viruses that we know? Notwithstanding a considerable uncertainty about any number that I may provide, we could say with reasonable confidence that the researcher could complete the visit to this part of the palace in just a few hours or so. In other words, the researcher could inspect the known part of the virosphere in just about the first hours of his or her life and therefore has the rest of it to enjoy encountering viruses yet-to-be described. This is where both virology and the virosphere exploration are at this very moment: just the beginning of a long, exciting journey.

Bioinformatics will be crucial for this journey, since practically all newly discovered viruses are known only as genomic sequences. Yes, we know blueprints of viruses and we must learn from these blueprints how these viruses function, how they are organized, how they have evolved, whom they infect, and whether they could cause any disease or be beneficial. It is why the examples I gave in my lecture will stay relevant for the future exploration of viruses.

One particularly important frontier of this work is virus taxonomy, as Andrew Davison and Stuart Siddell, who lead the International Committee on Virus Taxonomy may attest. In my group, Chris Lauber, PhD student at the time and a speaker at the Symposium today, and I developed a computational method that could classify viruses at different levels. This method produced sensible results for several virus families, and Anastasia Gulyaeva, Igor Sidorov, Dmitry Samborskiy and Andrey Leontovich in my group keep improving it and expanding its application. It uses software developed by the late Alexander Kravchenko.

The ongoing explosive advancement of our appreciation of the virosphere diversity will generate a giant wave of new knowledge in fundamental and applied research, which in all likelihood will reach the healthcare system soon. It will prompt a major transition in a way we approach viruses, and like any transition, it may be painful. On the other hand, "*If we want things to stay as they are, things will have to change*", according to nobleman Tancredi on another major transition, in the "Leopard", which I have cited before.

Ladies and Gentlemen, it is my pleasure and privilege to conclude this lecture with a few brief remarks and words of thanks

So why do I study viruses? Is it because I want to contribute to the fight against viral diseases in humans and other species we care about? Or is it simply because it just happened to me and I continue doing virus research because I know how to do it? Or is it because viruses are small, smart, beautiful and challenge my perception and knowledge, and I am curious? I must have felt differently about these questions at different points in my career, and it would probably be accurate to say that all these facets combined inspire my work now.

I feel that while I have been studying viruses, they kept teaching me about the world we live in. They connect us to people and other species through invisible infections, and it is they who connected me to you, my colleagues and friends who came from nearby and faraway on this occasion.

My initiation into virology prompted the first relocation of my family when my eldest daughter Yana was just 10 months old. Forty-three years later, and a hundred relocations in between, I am very happy to share this special moment with my youngest granddaughter Anastasia and her mother Sonya, and the twins Nora and Vera and their mother Yana and her boyfriend Michel, and my wife Olga. And I know that Sonya's eldest daughter Katya, the busiest one, is here in spirit.

Thank you colleagues, friends and family for coming. Thanks viruses and Leiden for connecting!

Ik heb gezegd

Further Words of Thanks

To Prof. dr. Willy Spaan. Willy, thank YOU for inviting me to Leiden for the first seminar in 1991 and to many other occasions and creating the opportunity to move to the LUMC in 2001 to continue my research here, and for the subsequent and continuing support in all your positions of high responsibility at the LUMC.

I am most grateful to Prof. dr. Pancras Hogendoorn and Prof. dr. Eduard Klassen, the current and former Deans of the Medical Faculty, for their encouragement and backing, especially of the collaborative Program on Bioinformatics between the LUMC and Moscow State University, known as MoBiLe. Initiated by Willy Spaan and Prof. Vladimir Skulachev, Dean of the Bioengineering and Bioinformatics Faculty of Moscow State University, and with the support of many LUMC departments and professors of MSU, it has provided a unique opportunity for the best Moscow students in bioinformatics to conduct ambitious projects designed by LUMC researchers. This Program has also supported software development for virus exploration in my group. It's been a privilege to be part of this Program.

I feel particularly honoured to have been a recipient of the Leiden University Fund Chair for eight years. And I would like to thank the governing bodies of the LUF, Leiden University, and the LUMC for establishing this Chair.

To Prof. dr. Louis Kroes. Louis, thanks so much for chairing the Curatorium of my LUF Chair, for supporting my group over the last ten years, and for the opportunity to work at the department of medical microbiology beyond my retirement age. Also, my profound thanks for the unique opportunity to hold a Symposium in relation to my retirement. Your generous support of the Farewell activities made this lecture and the entire gathering possible. Very Special!

I would also like to thank speakers and chairs of the Symposium, all my other collaborators, some of whom are here today, some of whom who unfortunately could not attend, and some of whom who are sadly enough no longer among us, for their generosity. It is an incredible feeling to share this moment with my mentor Vadim Agol, my lab mates from the different laboratories I worked in, my other esteemed collaborators, fine post-docs and students, especially, Danny and Danny, Siamaque, Chris, Kathleen and Anastasia, and also the colleagues who made my research possible, although they may have never been mentioned by name. Thank YOU everybody!