Could We Live in Peace with Viruses?

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Mijnheer de Rector Magnificus, hooggeleerde collegae, zeer gewaardeerde toehoorders.

Virus infections as a shared human experience

We enter this world and make our journeys in time and space that are unique in many respects. Yet, there are some experiences - few I must say - that unite us, human beings. Drinking water, watching sunsets and feeling pain are among these basics. Being universal, they are elevated to a special place in our personal and social conscience, records of our lives and become essential for us.

I would like to talk here about one experience that - without being broadly acknowledged - belongs to this exclusive list and leaves numerous marks in our life. I am talking about *viruses* or, more precisely, about *infections* they cause, which can be seen as our shared experience. During the next half hour or so I will explore how we perceive and study viruses. I will reflect on the advancement of our knowledge about viruses using my personal research know-how as a virologist and a bioinformatician, and by examining general trends. In the concluding part I shall summarize my understanding of what drives our exploration of viruses and how the accumulated knowledge is utilized practically. I will ask myself several questions about viruses. I believe that answers to these questions are relevant for our personal and collective choices in the everyday life, both the short-term and the long-term ones, and also for defining our goals for future virological research.

To start, may I reiterate something trivial: we are *all* infected by viruses. Everybody in this room and outside it, from the first to the last day of her or his life, and regardless *where* one was born, raised and lived - we are all infected. Whether one lives in a tropical forest, arctic tundra, African desert, on a small island or high in mountains, in New York or Leiden, and regardless of personal fortune or misfortune – whether one is exceptionally accomplished or physically underprivileged, and enjoying enormous wealth or struggling to meet ends - we are all infected. And we, humans, are not unique in this regard: all other species living on this planet – all animals, plants and single cell life forms that we use, care or are not aware about - are infected too. Furthermore many viruses infect more than one species, thus providing

unexpected links between sometimes very diverse life forms. This is what we observe today, this was true yesterday and this will be true tomorrow.

Although the picture of the pan-virus infection of the entire biodiversity that I have just painted sounded as most definitive, it is my personal take on viruses that may be not universally accepted. My conviction is based on the available data which are fragmented and may never be sufficiently complete to proof this vision to the last point.

Four Perceptions of Viruses

While viruses are everywhere, they may be perceived differently by public and in the research community. In total, I distinguish four perceptions of viruses that I am going to introduce one by one and in comparison.

Unlike most other universal human experiences, a virus infection may often go unnoticed. But when things, suddenly or gradually, start going wrong in our bodies, viruses may be blamed; often viruses are invoked because of the lack of other explanations. Fortunately, most of us *suffer* from virus infections only occasionally, with the first years of the life being a notable exception, as many parents will vividly recall. And this early experience is formative for our general perception of viruses: in our minds they forever become associated with unpleasant memories, and this feeling only grows with age and becomes more pronounced with our experience. In fact, this *negative* perception of viruses is very fundamental. It might easily be translated into a strong antivirus message and made part of the political message of somebody running for public office. Yes, an antivirus party – if ever created - would certainly enjoy a broad public approval.

People decide about viruses through personal experiences, and they associate viruses with disease while never observing viruses properly. That is because of the minuscule size of viruses, which kept them invisible until their presence was revealed in simple yet ingenious tests by Dmitry Ivanovsky, Martinus Beijerink, Friedrich Loeffler and Paul Frosch a century ago. Soon after viruses were discovered, the debate started about whether they are alive or not. Since viruses may not reproduce on their own and

require a living host to proliferate, some say they are not a life form. On the other hand, viruses have important characteristics that are otherwise found only in cellular life forms. These include the chemical composition of their building blocks, the usage of a genetic blueprint to program and direct reproduction, and their ability to adapt to environmental changes through evolution in generations. The ongoing debate about '*what is a virus*?' may never end, but there is little doubt that viruses form a biological class of their own. Consequently, they firmly belong to biology and are studied using the powerful tools and diverse techniques currently available in this branch of science.

These studies, using advanced techniques, like X-ray analysis and electron microscopy, and starting some 70 years ago have contributed to a second perception of viruses: they are tiny particles of different size, form and shape, often with symmetry that reveals repeating blocks from which they are built. The visual image associated with this perception is most striking. It is no wonder that it has started to dominate in communications targeted to research community and for public consumption. It is projected from posters, featured on cover pages of textbooks and journals, and engraved in logos of many organizations studying viruses. This image is also enduring: when the Dutch Society for Microbiology recently updated its society logo, some bright colors were added to the familiar image of a virus particle with spikes, which otherwise changed little compared to its original that was conceived decades ago.

We shall not overlook that viruses are also dissected by many other techniques, which continuously update our knowledge base about viruses. We have learned from these studies that viral life, if I may apply the word "life" to viruses, is a *cycle*, commonly including intracellular and extracellular stages. The common images of viruses that I have mentioned before are snapshots of the extracellular stage, which is called virion or virus particle. They may survive in the environment and be the first to be recognized by the immune system, when virus infects humans or other mammals, and they are the first to interact with the cell to initiate infection. Yet, the virus-host interaction is far more complex during the other, intracellular stage of the viral life cycle, when the virus has entered into the cell and starts to exert its influence directly and indirectly in many ways. Our understanding of this stage is poor, even for the best

studied viruses. So far, no common image of the intracellular stage of the virus cycle emerged that could shape its perception by public. This deficiency does not prevent many virologists from considering this stage to be the key for understanding viruses. Thus, the intracellular stage of the viral life cycle provides the basis for the 'third' perception of viruses, following those associated with disease and virus particles.

There is yet another, *genetic*, dimension, in which viruses are characterized. One of the classic disciplines, virus genetics entered a new era some 30 years ago. That time the first blueprint of all instructions of a virus to produce progeny – the *genome*, as we say – was decoded. Many others followed soon. Genomes exist and function as a chemical molecule, a polynucleotide, which can be either linear or circular. It is composed of four varieties of the same building block, the nucleotide. The order of nucleotides in the genome is known as the genome *sequence*. Virtually, we could separate the information in such a sequence from its polynucleotide carrier, and write it as a text using a four-letter alphabet consisting of the letters A, C, G, and either T or U to distinguish the two known polynucleotide varieties, DNA and RNA, respectively. Each letter in this text would correspond to a nucleotide. This genome sequence is an abstraction that captures the genomic information and, when written on paper, such a genome sequence is similar to any other text written in a human language. Since the start of the genome era, genome sequence characterization has been the fastest growing segment of our accumulating knowledge about viruses. From this genetic perspective, viruses could be perceived as programs written in a 4-letter code. For humans, this perception may not be very appealing for the two reasons: the virus text is written in a language nobody understands or speaks, and this text could be easily confused with those of cellular organisms.

Although, a connection between genomes and viruses may not be visualized in an easily accessible form, it is through genomes that we gain an insight into viruses that is unbiased, accurate and comprehensive, a combination that makes the genetic perspective, the fourth in my list, the most informative one. The accuracy of genome sequencing is approaching 100%; no other property of viruses is systematically characterized with a better quality. Since the cost of genome sequencing has been falling dramatically, nowadays the genome sequence has been determined for virtually every virus we know. Often it is the first information we collect about newly

discovered viruses, and for a growing number of viruses it is the only information available. The genome sequence can be easily transformed into a digitized form for efficient storage and analysis to reveal what it encodes, how the encoded products operate and function, and how genomes evolve. In summary, the genome sequence is an unparalleled source of information about all aspects of viruses: their pathogenic and non-pathogenic properties, as well as extracellular and intracellular stages of life.

Virus Bioinformatics – Powerful Approach for Reading the Genome Message

It would be wonderful, if we could read viral genome sequences like we read books and understand what they tell us. Unfortunately, as I have already mentioned, virus languages are so different from human languages that we need to learn them from scratch. During these efforts, we rely upon a fundamental observation about genome languages that also applies to human languages: the closer viruses (or languages) are in evolution the more similar their genome sequences (or vocabularies) will be. In linguistics, this relation has been exploited to recover lost human languages. In biology, genome comparisons are assisted by numerous tools that can assess the statistical significance of the similarities observed. Those similarities that are found to be statistically significant could be biologically sensible. The tools employed belong to the field of *bioinformatics*, a young branch of science that involves extracting biological information from genomes and their products. While applying bioinformatics, genome texts are broken into pieces that are either similar or not in the compared genomes. The most similar pieces have been selected in evolution to work in molecular environments that vary little in the life cycles of different viruses. Appropriately, they are called *conserved* sequences. Their conservation also extends to the function and the structure of products encoded in the respective pieces of these genomes. Likewise, pieces with little similarity are likely to be unique in terms of structure and function, and interact with elements of the environment that vary between viruses. Both conserved and non-conserved pieces may play biologically important roles, ensuring that the virus life cycle goes on as before, even in a changing environment.

Splitting a genome into conserved and non-conserved pieces does only part of the job on our way to understand what each piece is responsible for. It is only occasionally

that some understanding may be achieved at this early stage. Much more commonly, initial insights are gained upon the experimental characterization of a virus. Once this characterization is successfully accomplished, the established characteristic or, in other words, its meaning may be assigned to similar pieces in other genomes by genome comparison. This assignment thus comes down to a transfer of knowledge about one genome to another genome. It can be done across all related genomes, regardless of their origin, and may involve also cellular organisms. In the case of viruses, it is not restricted by the host of the virus either. Consequently, studies of non-human viruses may contribute to our understanding of human viruses, and vice versa. This is true even for non-human viruses for which no other information but their genome sequence is available: these genomes may assist in establishing similarities between viruses that may otherwise seem to be unrelated.

The way in which bioinformatics and experimental characterization interact has transformed research in biology, including virology. Importantly, bioinformatics does not merely provide an information network through which an initial experimental observation can be disseminated from one biological entity to others. Its benefits include considerable synergy and cost reduction in multidisciplinary research, and it can generate paradigm shifts in concepts that were formulated based on experimental research only. Let's consider a few examples.

From all disease outbreaks caused by a newly emerged virus species in the 21st century that of severe acute respiratory syndrome (SARS) was the most damaging. It claimed the lives of more than 800 people on two continents and resulted in considerable uncertainty about the future for several months, during which the daily life of millions of people was disrupted and huge economic losses were recorded by many businesses and governments. The virus causing SARS was rapidly identified as a new coronavirus. That identification helped to focus the search for a cure on developing drugs that could selectively target SARS-coronavirus. A candidate compound was identified almost momentarily and described within weeks in a Science publication: it was an inhibitor of an enzyme called *protease*. That enzyme controls the execution of the SARS-CoV blueprint instructions or, as researchers like to say, it controls the virus genome expression. Until the outbreak was contained through quarantine, this inhibitor was the most promising lead to develop a drug that

could be prescribed to humans. The history of its development, which I would like to share with you, highlights the various ways in which virus bioinformatics can contribute to the advancement of fundamental research and its applications. For me it has also a personal dimension and connects my work in the biochemical lab during my PhD project to subsequent bioinformatics analyses that were conducted over some 20 years.

This story begins more than 30 years ago when biochemical studies identified another protease as a key enzyme of a mouse virus, a model virus for the human poliovirus. This enzyme was subsequently shown to be conserved in the entire family of picornaviruses, to which the mouse virus belongs. Applying bioinformatics, a new discipline in those days, a biologically sensible link between the proteases of picornaviruses and enzymes operating in human organs, like the pancreas, was unexpectedly established. It enabled the modeling of the tertiary structure of the viral protease and the mapping of its most critical elements. This analysis revealed that picornavirus proteases combine properties, including their tertiary structure and active site residues, that were considered incompatible by researchers at that time. Prompted by this discovery, picornavirus proteases were characterized by many techniques, including X-ray analysis. The results obtained fully validated the initial observation and supported the revision of a paradigm, one of the biggest in studies of proteases.

In parallel with this advancement, bioinformatics identified a similar protease and its substrates in a bird coronavirus of which the genome was the first to be fully sequenced. This identification was particularly insightful, since it also implied that proteases control coronaviruses and picornaviruses in a very similar manner, by recognizing similar signals in substrates. Such a profound similarity concerning a major characteristic came as a big surprise to researchers. In prior studies, they found little in common between these virus families, apart that they all use a particular type of the genome molecule known as positive-stranded RNA. At the time, two coronaviruses were known to infect humans, but both were treated as a minor public threat because of a mild disease that they caused. Due to the lack of a major human pathogen among coronaviruses and technical challenges, the characterization of the coronavirus protease was pursued by only few groups at the time. These studies progressed steadily over more than a decade. During few years at the border of the

20th and 21st centuries this effort was mainly driven by the curiosity of Dr. John Ziebuhr who wanted to gain insight into the properties that distinguished the proteases of coronaviruses from those of picornaviruses, as was apparent from bioinformatics analysis. His persistence was rewarded when the tertiary structure of the protease of yet another coronavirus, the one infecting pigs, was solved through collaboration with the group of Dr. Rolf Hilgenfeld and published just months before the onset of SARS-CoV outbreak. This structure enabled the transfer of the accumulated knowledge about inhibitors of the activity of this type of protease from picornaviruses to coronaviruses. It enabled molecular modeling of substrate-like inhibitors. That aspect emerged as critically important for the development of drugs active against SARS-CoV, after the human population was confronted with a challenge to control the outbreak caused by this virus.

The story that I just told you was probably lengthy. This might be justified if it has given you a sense of the involvement of bioinformatics in a multidisciplinary study that was conducted by multiple laboratories over the span of some 35 years. Due to the focus of this lecture, it is naturally centered on the contribution of bioinformatics to reveal it fully. In my opinion, three major lessons can be drawn from this story. First, bioinformatics analysis can stir a field to conduct a new and very productive line of research that may lead to a radical revision of a prevailing point of view. Second, although the resolution of bioinformatics analysis is limited, its results may be as reliable as data produced by experimental research; in this respect they are observations, rather than predictions as many would call them. Third, the full benefits of bioinformatics insights can be harvested upon the interaction of bioinformaticians with experimental researchers, when the strengths of each approach are combined to generate the kind of synergy that leads to major advancements.

May I take few more minutes of your time to show that the bioinformatics contribution is continuous and its insight can be extended to the future like it was traced back in the past.

I will illustrate this point by starting from the place where I stopped my first example: the SARS-CoV genome. The bioinformatics-based identification of the protease that might serve as a target for the development of anti-SARS therapy was a routine

exercise at the time. However, the comparative analysis of the SARS-CoV genome we conducted at the time also produced deep insights into coronaviruses that impact our understanding of the entire class of RNA viruses to which coronaviruses belong. To appreciate this insight, let us recall that the principal characteristic of RNA viruses, which distinguishes them from DNA viruses, is the fact that they are incapable of correcting mistakes made during genome amplification, or replication as researchers say. The consequences of this deficiency are profound. First, RNA viruses are severely restricted in the size of their genome, and thus their tool-kit for adaptation is limited. Second, they proliferate as clouds of mutant forms, the property that determines the way in which they adapt to new niches and escape from immunity or drug therapy.

Well, the bioinformatics analysis of the SARS-CoV genome showed that this theory may be not fully applicable to coronaviruses, which are RNA viruses as you remember. It showed that coronavirus genomes encode a counterpart of a cellular enzyme that corrects replication mistake and thus promotes a high quality of replication, or high fidelity as researchers say. This enzyme, a so-called exoribonuclease, was also identified in other RNA viruses, all of which are distinguished by having relatively large genomes. This initial insight has now been validated by several groups in studies involving biochemical and reverse genetics characterization of the enzyme. This characterization is continuing and we expect to learn how coronaviruses use this enzyme to foster their adaptability in a way that may deviate from that described for other RNA viruses.

Our Future with Viruses

We, humans, must take a lesson from viruses if we want to adapt to our changing world. As we have learned – particularly over the last decade or so - the world is indeed changing and changing fast because of us. We are now confronted with multiple challenges to adjust our personal choices and our collective interactions with the environment. The main focus is often about choices we make concerning energy, its production and consumption, and the impact of these choices on the global climate. Many other things, big and small, appear to be secondary to these choices. The truth, however, may be more complex since we impact the world and our own well-being

also through interactions not involving energy. Choices made there are numerous and they must also be sustainable. They include interactions with viruses that have so far been shaped by the negative perception linked to the disease and death viruses cause. I believe that this dominant perception must be reviewed for our own good and extensive genetic characterization of viruses may help in this quest.

In the public eye, viruses are portrayed and seen as enemies, and the good enemy, as the saying goes, is a dead one. This is the notion on which our long-term strategy to deal with viruses was originally built and from which it is being advanced now. We employ many fine tools to detect viruses, and prevent, cure and contain virus infections. The funding priorities for research on viruses are determined by these practical considerations: the development of sensitive high-throughput methods of virus detection, potent antivirus drugs and prophylactic vaccines, all to better equip our health care system to control or fight virus infections. Our traditional approaches to deal with viruses are now extended with the bold vision of a virus-free world, in which we are liberated – forcefully of course - from the viruses that bother us most. This vision is rooted in the success - truly remarkable in many respects - achieved with the eradication of a single virus, the human poxvirus, which devastated human populations in the past. The same vision has been applied globally to get rid of another virus, poliovirus that caused pandemics. In this case, the campaign has been going on for the last 25 years and is well beyond its original deadline. Although this delay might serve as a warning that the initial success with the poxvirus could be an exception, other viruses wait in the pipeline to follow, and the temptation grows to proceed with the next virus anyway. We *are* the Masters of this world and we can decide who can live and who must go.

Shall we follow this path that seems to lead us to a sort of safe, albeit a bit sterilized future? If you wonder why I don’t embrace this future right away, here are some questions for which I do not have answers. We know that many viruses, classified as pathogenic and circulating in the human population, cause fatal or other serious complications in a minority of infections. It is true that even small percentages could translate into large numbers of victims when the number of infections involves many millions, and this is why we mobilize our resources to bring down these small percentages. Yet, we may not escape the question what these infections cause -

particularly in the long term - to others, the *majority*, who were infected but did not suffer noticeably? A similar question could be posed in relation to those viruses whose infections are resolved without any visible sign to *all* infected: are these infections bad, good or something in between? Would it be better to learn first about a virus before trying to remove it, through eradication, from an ecosystem containing balances and intricacies that we may barely understand? Eventually these and other questions we could ask concern the long-term impact of virus infections – past, present and future - on us, human beings, as individuals and as a species: do these infections affect our personal and collective fitness? And, to make sure that we speak one language, fitness is about our survival in a changing environment for generations to come.

We have learned that human viruses form a large and extremely heterogeneous group, including all kinds of mini-creatures that may be as distantly related to each other as, say, humans and *E. coli* bacteria. So, despite being similarly small, they are very different. Likewise, virus infections could be as diverse. After entering the body by one of many possible routes, the virus may either generate a large progeny and leave the infected host promptly; or alternatively, keep low in the body for as long as the host hangs around. With the spectrum of infection strategies ranging from the one extreme case to the other, and considering the hundreds of diverse viruses that we know to be around, it is likely that virus infections directly or indirectly interact in humans. What happens upon this interaction is a big unknown. Is it important to learn more about these interactions before deciding to start the eradication of a virus or before claiming that eradication of a virus could be nothing else but a success?

Viruses also infect other species and they can “jump” from one species to another. This happens occasionally, like we observed with SARS coronavirus, or regularly, like we experience with influenza virus. The virus jumps to a non-infected human either upon direct contact, or through exposure to a virus in nature or through intermediate hosts, ‘vectors’ as virologists say. These types of virus transmissions that cross barriers between different host species are most threatening. It is this type of interactions that makes the headlines for all possible bad reasons, including high mortality and uncertainty about the future, to name few. Could these complications be avoided or, at least, made less painful?

How we manage our relation with the influenza virus may be used as an example of a thoughtful approach to human-virus interactions. We study the influenza virus very carefully to anticipate its future changes. This provides us with a time window to adapt our individual and collective behavior in order to minimize the negative impact of the infections that follow. Probably we would love to eradicate the influenza virus and forget about it for good, but we may not accomplish this task: the virus has a large reservoir outside humans which we can not destroy without undermining seriously our own affairs.

This model does not work for all viruses, particularly those newly emerging viruses that infect us for the first time or only occasionally. Each time when a newly emerged virus is discovered it comes as a surprise. It reveals the scale of our ignorance about the viruses circulating in the neighborhood in which we live, and which we probably disturb. This knowledge gap could be closed with a systematic description of the genomes of all virus species and virus habitats, like it is being done with other living beings. It is a gigantic effort, but it may be worth it.

Genome sequences of the Virus World around us could be systematically analyzed by bioinformatics. Because of the huge number of genomes that could be made available for analysis, we may expect a considerable advancement in our understanding of the virus evolution, function and structure. My group is working toward developing a genetic-based approach to virus classification that utilizes genome sequences. It could be instrumental for linking virus taxonomy with fundamental and applied virus research. This area is rich for the research directions that could be explored with the involvement of motivated talented students. These students could come from within LUMC and from outside, particularly through the Joint Program in Bioinformatics that was created between LUMC and Moscow State University, Russia. Eventually we could set ambitious goals of estimating potential of viruses to be harmful for us and conditions under which the harm may be inflicted. This knowledge would empower us in making informative and sustainable choices on different levels, including the use of traditional tools, like vaccination and drug therapy, and also modifying our behavior if it is necessary.

As we all know well, the life is a dangerous affair. We accept this notion, and some people even develop a sort of admiration for sources of the danger, like cobras or cars to name few. Our long term approach to these dangers is to minimize risk involved and maximize benefit. We apply this approach to many biological species and we could extend it to include viruses. After all, we and viruses share this world that depends on viruses as much as it does on us.

Words of Thank

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I gratefully acknowledge generous support of my research and teaching in Leiden by the Department of Medical Microbiology, which is a part of the Center of Infectious Diseases.

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I was very fortunate with having been admitted by Prof. Vadim Agol to his famous lab at the Institute of Poliomyelitis in Moscow soon after my graduation from Novosibirsk State University some 35 years ago. In the Agol's lab I learned, for the first time, about the world of viruses and met brilliant researchers who set standards of excellence in many dimensions that I was not even aware about. I started my personal exploration of the virus world with experiments on a mouse picornavirus that were initiated under the guidance of Dr. Konstantin Chumakov, and later conducted in collaboration with Dr. Yuri Svitkin. Through the formative 20-plus years of the association with the Agol's lab I earned my degrees and developed my approach to study viruses. This advancement proceeded in the intellectual environment in which

the excellence in making scientific inquiry and dissecting obtained results were the primary and tough challenges to meet. My mentor has been and remains a step ahead in this endeavor, each year setting the bar a notch higher, and it is an exceptional honor to have Prof. Vadim Agol here at this occasion.

My first exercise in, what is now called bioinformatics, was applied to the freshly released poliovirus genome – one of the first for viruses - during final years of my PhD study. Vladimir Blinov, late Alexey Donchenko, and Dr. Eugene Koonin with their enthusiasm, dedication and complementary expertise transformed this one-time curiosity inquiry into a life-time line of research. The perspective which I've shared with you today is routed there.

Prof. Michael Lai was the first to trust a newly sequenced virus genome, that of mouse hepatitis virus, to me for bioinformatics analysis. I still remember huge excitement of that 20-years old study.

Prof. Michael Rossmann and Prof. Stuart Siddell hosted me for extended periods of time in their laboratories at Purdue University, W. Lafayette, USA and Wuerzburg University, Germany, respectively. It was enlightening to collaborate with the groups working at the forefront of structural and reverse genetics studies of viruses.

In Wuerzburg, I got acquainted with Dr. Jens Herold and Prof. John Ziebuhr, young researchers at the time, with whom we later collaborated and who introduced me to Germany I did not know about. Getting a glimpse of the freshly solved structure of the first coronavirus protease was a gift from John I may not forget.

Thanks to the invitation of Prof. Don Summers, I enjoyed the opportunity of working at a world premier biocomputing center in Frederick, MD, USA for four very productive years. Very sadly, Don is no longer with us.

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To Prof. dr. Louis Kroes. I am most grateful to you, Louis, for your continuous support of my group at these challenging times and for your enthusiasm about new projects that we are trying to pursue in connecting fundamental research to clinical practice. Special thanks for your constant encouragement to proceed with this lecture.

To people in my group. It's been a privilege working with you. You teach and liberate me.

To my friends: I am a rich man because of you.

My journey in this world was started by my parents, Yevgeniy and Sofiya, who remain in my heart. I continue it with my wife Olga, who somehow makes the big virus world looks small compared to that of my family. I am very happy to see that

my children, Yana and Sonya, and those who they love are trying hard to find their way. And I wish my grandchildren will ask and answer their own questions that could help make this world of viruses and men a better place to live in.

Ik heb gezegd