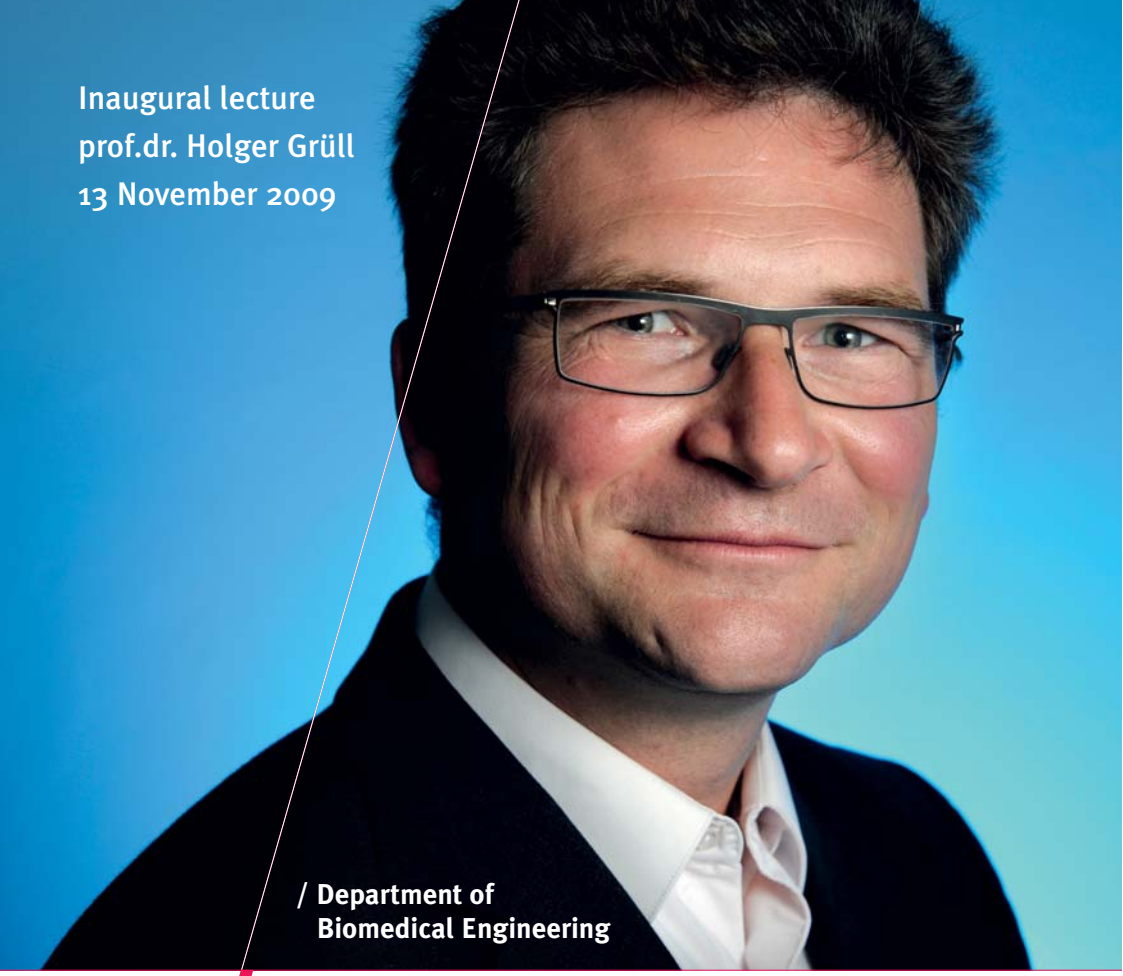
Insights from inside

Inaugural lecture
prof.dr. Holger Grüll
13 November 2009



/ Department of
Biomedical Engineering

TU/e Technische Universiteit **Eindhoven** University of Technology

Insights from inside

Where innovation starts

Inaugural lecture prof.dr. Holger Grüll

Insights from inside

Presented on 13 November 2009
at the Eindhoven University of Technology

Introduction

Molecular imaging and therapy

In my inaugural lecture, I would like to present the research I am doing in the field of Molecular Imaging and Therapy together with students and colleagues at the Eindhoven University of Technology and with colleagues at Philips Research. An inaugural lecture does not have the intention to be a scientific talk, but rather to introduce the ideas and trends of the respective area of research to a broader audience. My chair focuses on new nanostructures for applications in Molecular Imaging and Therapy. Why nanostructures, what is so special about them, especially for their use in Molecular Imaging and Therapy, that we devote a group to work on this topic?

Today, medical imaging systems like Computed Tomography (CT), Magnetic Resonance Imaging (MRI), Ultrasound (US) and Nuclear Imaging are standard techniques in the clinic to provide the radiologist with pictures of the body to diagnose and stage diseases. Currently, diagnosis of a disease is largely based on morphological changes within the body, which can only be visualized with above mentioned medical imaging techniques at a rather late stage of the disease. In order to allow earlier and better diagnosis, contrast agents have been developed for all imaging modalities to enhance the difference between healthy and diseased tissue.

We are working on a new generation of agents based on nanoconstructs to be used with imaging modalities and for therapeutic application. Nanostructures can be designed at length scales ranging from nanometers to microns and out of different materials with unique functionalities; combined with contrast agents they provide a signal in the body that is observable with one of the above mentioned diagnostic imaging modalities. Nanoconstructs may be functionalized with targeting ligands to specifically target diseased cells (Figure 1). Injected into the blood stream, they interact with a living being at the molecular, tissue or organ level, thereby enabling new applications in the clinic, but also serving as probes to investigate and visualize biological processes. These applications are called molecular imaging and may allow for more specific and better diagnosis of diseases.

Nanoconstructs may also carry a drug-payload that they deliver locally to diseased cells and tissues. They do this either by reacting to the environment present only in diseased tissue, like a different pH or specific enzymes, or by reacting to an external trigger, for example a subtle temperature increase in the diseased tissue induced by external heating.

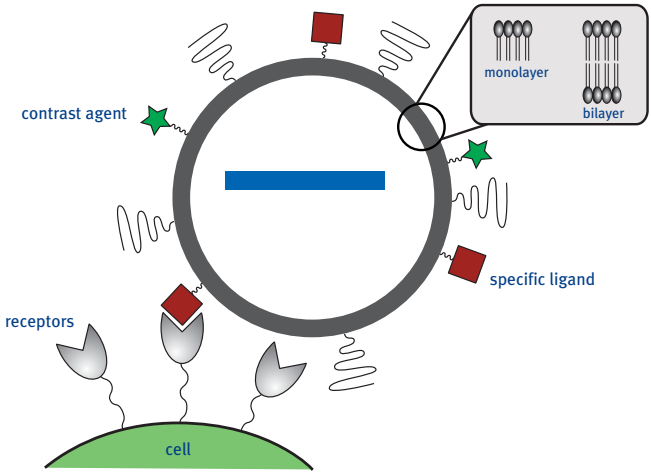


figure 1

Nanoparticle (diameter ca. 100-200 nm) carrying contrast agents (green stars) and a drug payload (blue) binds to cell surface receptors of a diseased cell via a specific ligand (red squares).

Research in Molecular Imaging and Therapy is a broad and multidisciplinary field: it starts with *insights* into biological processes and diseases, it requires chemistry and radiochemistry, biology, preclinical studies using diagnostic imaging to 'look' *inside* and see what is happening, and last but not least a thorough analysis and interpretation of the data. This research serves its own purpose, namely to increase the knowledge of biology and diseases in preclinical models: how molecules and particles interact with the living being once injected into the blood stream, and how to use the above tools to interrogate a complex living system to get a read-out of biological functions. This type of science is devoted to understanding fundamental mechanisms and to generating knowledge. If we go one step further towards clinical applications, the translation of the above work into the hospital has its own challenges and comes with a different set of requirements. Research becomes application driven. Developing new applications requires research to generate the necessary *know-how* in order to turn scientific *knowledge* into products.

I spend a considerable part of my time as a researcher in an industrial laboratory, with the aim of doing research and development that will eventually lead to new medical products. With my assignment as a part-time professor in academia, my research gained a second aspect. My academic research is well aligned with the application we are working on in industry. Yet, academic research has to meet different rules and serves other purposes than developing products. In the second part of my talk I will discuss in more depth the different aspects of academic and industrial research, and their implications for the education of students.

I will start off with a brief introduction on dimensions and length scales in biology that are of relevance when we consider different concepts for research in vivo, followed by three examples of research in Molecular Imaging and Therapy. I will show how we can use molecular imaging to acquire new *insights* by looking *inside* a living being.

Length scales in medicine and biology

From atoms to organ

Medical and biological research deals with phenomena at many different length scales, comprising among others molecular interactions at the nanometer scale, and cellular interactions at the micrometer scale. While the root causes of diseases are aberrations at the molecular level, many diseases only become noticeable once morphological changes occur at tissue level. Tissues and organs have dimensions extending from millimeters to centimeters, while the human body as such is on a length scale of a meter (Figure 2). Matching these length scales, most clinical imaging systems currently allow us to visualize structures with millimeter resolution.

Over the years, for all imaging technologies, contrast agents were developed that enhance the existing contrast. For example, MRI and CT contrast agents make use of compounds based on gadolinium or iron oxide nanoparticles, or iodinated molecules, respectively. For US, stabilized tiny air bubbles can be applied. Nuclear imaging is the only modality that always requires agents. Here, radiolabeled compounds are injected and pictures are acquired based on the emitted radiation. The size of agents for each imaging modality differs: clinically approved contrast agents for MRI, CT, and nuclear imaging are small molecules with sizes around 1 nm that are rapidly excreted via the kidney. The size range of 5 nm to 5 µm is of great importance when it comes to injecting agents into the bloodstream. The size of 5 nm approximates to the cut-off for renal filtration; compounds below this size are excreted from blood into the urine, while compounds larger than 5 nm will eventually be taken up in the liver. This different clearance pathway has medical implications, as compounds taken up in the liver can induce severe toxicity. The upper limit is determined by the capillaries in the lung that have a diameter of roughly 5 µm, allowing red blood cells to just squeeze through. Anything larger than 5 µm may clog the arteries and induce a lung embolism (Figure 2). Currently, only two agents that qualify as nanoparticles are clinically approved for human application. For MRI, magnetic nanoparticles with sizes around 20-40 nm find application as T_2 contrast agents. In ultrasound imaging, air bubbles stabilized by lipids or polymers with sizes up to 2-4 µm are used as contrast agents.

My research is devoted to developing new contrast and imaging agents that allow more specific and therefore earlier detection of diseases. These new agents accumulate in the diseased tissue due to interactions with disease-specific molecules, for example certain receptors or membrane proteins that are over-expressed on diseased cells (Figure 1). These interactions are short-ranged with length scales between 1-10 nm at the most, based on electrostatic, hydrogen-bonding, hydrophilic-hydrophobic, sterical and entropic effects. Controlling these interactions allows us to make specific agents, and to change biodistribution or pharmacokinetic behavior. However, we are still far away from understanding all interactions of complex materials within a living being.

Take for example a small nuclear imaging agent with a size of around 1-2 nm, for example a radiolabeled peptide that specifically binds to a tumor cell. On injection into the blood stream, it will circulate and probably be excreted via the kidney due to its small size, but it will also be taken up in a tumor cell. Next, we use nuclear imaging, such as single photon emission computed tomography (SPECT) to localize the imaging agent in order to find and stage the tumor. Unfortunately we

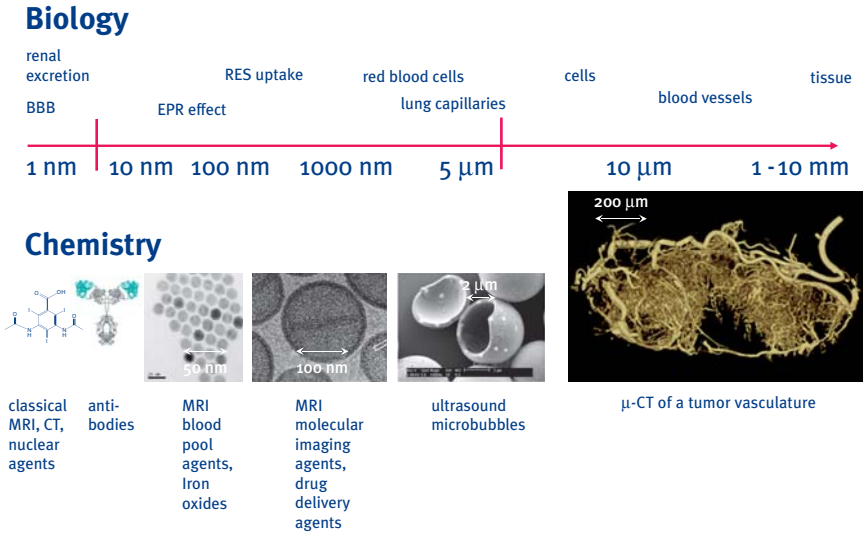


figure 2

Length scales in biology and medicine related to agents. BBB: blood brain barrier; EPR: enhanced permeability and retention; RES: reticuloendothelial system. Pictures below from left to right: iodinated x-ray contrast agent, iron oxide nanoparticles for MRI, doxorubicin-filled liposome for drug delivery, polymer-shelled microbubble for ultrasound imaging, micro CT imaging of blood vessels in a tumor.

cannot detect a single tumor cell, simply because the detection limit and sensitivity is not sufficient. With today's detection limit, only tumors of about a 0.5 cm-1 cm size are detectable, consisting already out of 10^9-10^{10} tumor cells. The development of better technology comprising more sensitive imaging systems and even more specific agents is therefore crucial.
Designing these new agents requires a thorough understanding of interactions in biological systems and the relevant length scales in vivo. Imaging techniques enable us to get to an understanding of how agents behave in vivo and to probe biological interactions.

Molecular imaging and therapy

From diagnostic imaging to therapeutic interventions

Iodinated nanoparticles for spectral computed tomography

Like so many important discoveries, the field of diagnostic imaging started with a coincidence. 114 years ago, Wilhelm Conrad Röntgen noticed some fluorescent light coming off a screen on his lab table. Measurements showed that this fluorescence was induced by then unknown rays emitted from a cathode tube nearby. This discovery is a remarkable point in history: x-rays were a new sort of radiation, which almost immediately had a high impact on research and led to applications. Röntgen used this new radiation to take the first diagnostic image ever. The x-ray photo of his wife's hand marked the birth of diagnostic medical imaging (Figure 3). In medicine, his discovery served many patients and presented the medical industry with a business opportunity that right up to today has been the cornerstone of Philips' business in computed tomography (CT) and x-ray systems. Working in academia and honoring his conviction that mankind should benefit from academic research, Röntgen refused to file a patent on his invention. As we can see in this photo, x-rays provide excellent contrast for bones due to the strong absorption of electrons in bones, mainly by calcium. Unfortunately soft tissues, like blood vessels, organs and muscles, are difficult to observe.
To overcome this limitation, people started to investigate iodinated molecules that strongly absorb x-rays once injected into the blood stream (Figure 3). It took another 50 years before the first iodinated contrast agent was developed for routine clinical use. Contrast agents added a whole new dimension to medical exams and possible applications, as suddenly blood vessels and soft tissue became visible allowing the diagnosis of many diseases.

Over time, the need for better diagnosis has been an important driver for new technical developments such as improved hardware but also better contrast agents. Today, with modern computed tomography systems and an iodinated contrast agent injected, it is possible to take movies of a heart within a few heartbeats.

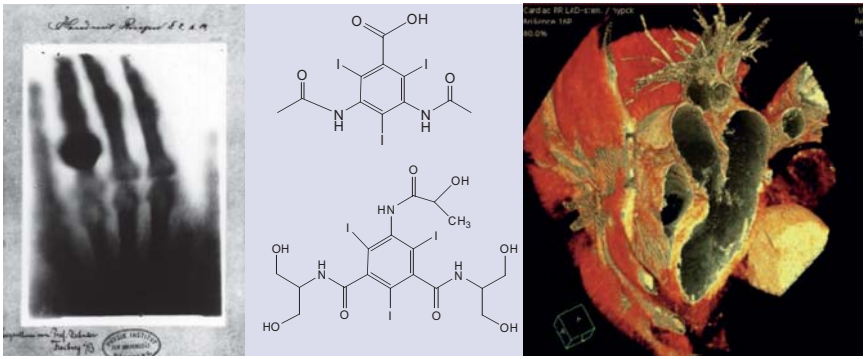


figure 3

Left: the first x-ray photo, taken in 1896, showing the hand of Anna-Berthe Röntgen;
center top: the first x-ray contrast agent approved in 1953 for human use;
center-bottom: modern x-ray contrast agent (iopamidol); right: CT scan of the heart.

Currently, there is one drawback to CT. It uses an x-ray source that generates a whole spectrum of x-rays. The x-rays cross the body and are partly absorbed or attenuated. A detector picks up how much of all the x-rays is actually absorbed. This method generates pictures purely based on the attenuation of x-rays; it cannot differentiate the source of attenuation. Thus a bone, or a blood vessel filled with iodinated contrast agent, all generate similar contrast and cannot be distinguished. By looking at the attenuation of light passing through materials, it is simply impossible to tell anything about the nature of the material. However, looking more closely, the attenuation of light does depend on its energy; this is why objects have a color, and the very same holds for x-rays. If a detector is used that actually resolves the energy of x-rays, it is possible to measure the attenuation of x-rays in computed tomography as a function of energy. As every element, especially heavy elements like iodine, strongly absorbs x-rays of a characteristic energy ('K-edge'), such a detector system can now differentiate whether absorption was caused by bone (i.e. calcium), or for example by iodine of a contrast agent. This technique allows us to see rainbow colors within the so far grayscale pictures of computed tomography. Measuring the absorption of the different 'x-ray colors', i.e. x-rays of different energies, enables us to identify and quantify different elements. Our colleagues at Philips Research Hamburg are currently developing such a new CT system, termed spectral CT, having an energy resolving detector [1]. In principle, this system should be able to provide an in vivo quantification of iodine in different tissues, based on the characteristic absorption of x-rays. In order to test this hypothesis, the iodine concentration determined by spectral CT needs to be cross-checked using an independent and established technique.

Here, multifunctional nanoparticles come into play that stay in the blood pool and circulate considerably longer than existing contrast agents. This agent is based on an emulsion of a hydrophobic iodinated oil stabilized by an amphiphilic di-block polymer, polybutadiene-polyethylene oxide. The hydrophobic polybutadiene

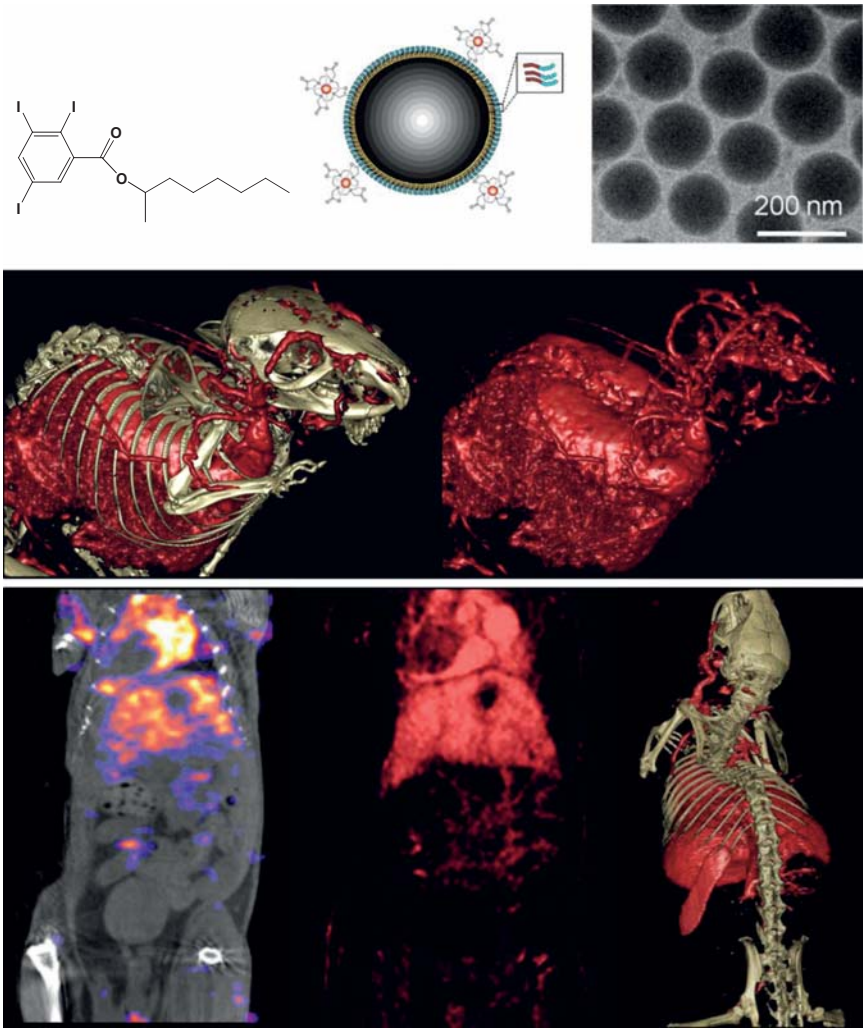


figure 4

Top left: iodinated hydrophobic oil incorporated into an emulsion (top middle) of approx. 150 nm; top right: cryo-TEM picture of emulsion droplets; center: spectral CT image with iodine concentration based image in red; below left to right: SPECT/CT, iodine picture obtained with spectral CT, and volume rendered spectral CT image of a mouse.

encapsulates the oil, while the hydrophilic polyethylene oxide forms a polymer brush extending into the water (Figure 4) [2]. When injected into an animal, these particles having sizes of around 150 nm are too big to leave the blood vessels (extravasate) or to be excreted via the kidney. The polyethylene coating leads to a long blood circulation time in vivo, as it shields the particle from interacting with macrophages. Also in conventional CT imaging these particles give a superb contrast: however, the contrast coming from bones and agents looks alike for the reasons explained above. The same animal can be imaged on a spectral CT system equipped with an energy resolving detector which now provides not simply contrast but gives color-coded pictures of iodine concentrations in different tissues (Figure 4). With spectral CT we can now distinguish contrast coming from bone and contrast coming from iodine to obtain impressive pictures of the vasculature where our blood pool agent is still circulating (Figure 4, center) [2].

Can these iodine particles also be used to get an answer if the new method in fact allows quantification of iodine? To shed light on the reversed question, the nanoparticles were labeled with a radionuclide, in this case Indium-111, by tagging it to the polyethylene glycol chain of each particle. As the radiation is proportional to the concentration of iodine, Single Photon Emission Computed Tomography (SPECT) can now be used to scan the very same animal and quantify the radiolabeled emulsion particles, and subsequently to calculate an iodine concentration based on the nuclear signal. We have obtained some preliminary results using this approach, in which we compare the biodistribution obtained with spectral CT with that obtained with SPECT (Figure 4). Interestingly, we observe a slight mismatch in the iodine biodistribution depending on the acquisition settings of spectral CT [2]. This approach of using an agent that can be imaged with both imaging systems allows us to improve a new technology. Currently, the study is still ongoing, but I hope that this experiment shows that new multimodal agents are not only a key to enable new applications, but they can also serve as a tool to test scientific questions.

Dual isotope imaging

Nuclear methods in medicine are almost as old as the application of x-rays. Shortly after Röntgen's discovery of x-rays, γ-radiation was discovered by Henry Becquerel. Radiation and radioactive materials were further investigated by Pierre and Marie Curie. Pierre Curie did the very first medical experiment to investigate the effect of radiation on humans by taping radium onto his own skin and watching how long it took to develop skin burns. Detection of thyroid cancer is in fact the oldest application of radionuclides in medicine, dating back to the

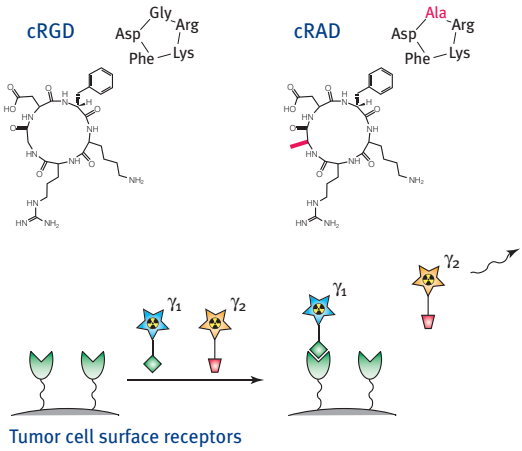
1940's, with radioactive iodine being injected into a patient to detect and treat the disease – a method that is still used today. Nuclear imaging developed rapidly with the invention of better and more sensitive detectors and different radioactive agents that today allow diagnosis of several types of cancer, but also provide functional information on organ perfusion, renal excretion and so forth. Recently, targeted nuclear agents were also approved. These agents consist of a molecule, for example a peptide or an antibody that binds specifically to molecules present on cancerous cells. This so-called targeting ligand is labeled with a γ-radiation emitting radionuclide that can be imaged using the nuclear imaging method SPECT. For labeling a variety of different radionuclides can be used, like Technetium-99m, Indium-111 or Lutetium-177. On clinical images obtained with these methods, hotspots indicate an accumulation of the respective radionuclide and point to abnormalities. In most clinical applications, only one radionuclide is injected, therefore hotspot, or grayscale pictures are enough for a diagnosis. However, SPECT imaging systems have energy-resolving cameras and allow distinguishing radionuclides based on the energy of the respective emitted radiation. Each radionuclide emits light of a characteristic color that can be visualized by a SPECT camera. Once two different molecules are injected – each labeled with a different radionuclide – and the color information can be used to differentiate the two molecules and visualize their individual behavior in the body (Figure 5).

What are the potential applications? Today, the clinical SPECT imaging world is largely a grayscale business as in most applications only one isotope is used. Only few 'dual isotope' applications exist, where two different agents each labeled with a different radionuclide are administered, for example in heart perfusion studies with Tl-201 and Tc-99m based agents to provide information about the heart at rest and under stress. Here, I would like to present an example, in which we use dual isotope imaging as a basic research tool to 'probe and interrogate' a living system to learn about molecular interactions in vivo.

For molecular imaging applications, the challenge is to design agents that home in on disease-specific targets which are over-expressed on diseased cells, for example tumor cells. The specificity of these agents comes from a strong intermolecular interaction of the targeting ligand with the target molecule on the cell. Designing such a target-specific agent is difficult. It requires a target-specific ligand like an antibody or peptide and its subsequent radiolabeling. The latter can again disturb the specific molecular interactions. In the first instance, the binding can be optimized in vitro using cell tests until specific binding is obtained.

However, only the in vivo experiment can reveal if specific binding and uptake in the tumor occurs. Many parameters intrinsic to the in vivo situation may prohibit tumor targeting, such as suboptimal biodistribution, like too short blood half-life due to rapid excretion, uptake in organs, metabolism, or non-specific interactions with other molecules present in a living being. Once an agent shows uptake in the tumor, it still has to be compared with an agent that is chemically similar but should not bind to the diseased cell to differentiate specific from non-specific uptake. A typical approach is to design a 'negative control' agent in which the intermolecular interaction between the targeting ligand and the target is strongly reduced by a chemical change within the binding entity. In the example shown here, the specific ligand is a cyclic peptide cRGD that binds to receptors present on tumor cells and angiogenic blood vessels in tumors. As a negative control, one hydrogen atom within the binding site is replaced with a methyl group, turning the amino acid glycine into alanine. On paper this looks like a minor change, in fact barely visible when looking at the chemical drawing of the molecule (Figure 5). However, changing the binding may not only change the intermolecular interaction with the respective target molecule, but can also change the overall chemical nature of the agent. The latter may lead to a set of different interactions with other molecules resulting in different in vivo behavior. From an epistemic point of view, this hypothesis can only be tested experimentally by changing the agent while keeping all other experimental boundary conditions constant. However, our experimental boundary conditions are defined by a living being and not by a well-controlled physical set-up. A living being presents a huge set of inaccessible and even unknown variables that, even worse, may differ from animal to animal.
Our experimental environment is therefore not constant but can only be approached statistically, hoping that the variations will average out once a similar experiment is performed in many animals. In a traditional way of comparing the positive and negative agents, their biodistribution and in vivo behavior is assessed separately in two groups of animals. The specificity is statistically tested by comparing the uptake of the two agents in the tumor. Dual isotope SPECT allows a very different and more elegant approach. Here, the two nanoconstructs are labeled with different radionuclides and are injected into the very same animal, thus keeping the external experimental parameters unchanged [3].
With this trick, we give each molecule a distinct color that allows us to observe its distinctive fate once injected into the body. In the experiment shown here, the peptide cRGD specifically accumulates in the tumor while the peptide cRAD does not, which can be seen nicely from their respective colors (Figure 5). Exchanging the radiolabel, in other words exchanging the color code, gives the same result, demonstrating that the radiolabel itself does not affect the biodistribution of the

peptides. Both peptides are excreted via the kidney as can be seen from the two colors showing up. With SPECT imaging, it is now possible to follow and quantify the biodistribution of each agent within the same animal, eliminating all possible inter-animal variations [3].



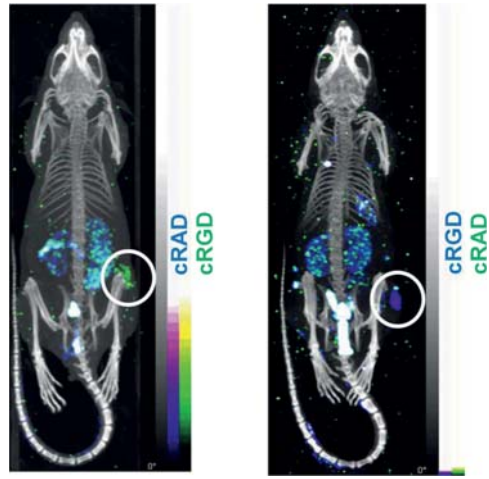


figure 5

Above left: cyclic peptide cRGD that specifically binds to the receptor $\alpha_v\beta_3$; above right: cyclic peptide cRAD as non-specific control. Center: cRGD and cRAD are radiolabeled with different radionuclides, cRGD is supposed to specifically bind to a cell surface receptor and cRAD is not. Below: dual isotope SPECT scan of a tumor (white circle) bearing mouse, in both cases the specific agent cRGD is taken up. Left: labeling of cRAD with In-111 (blue) and cRGD with Lu-177 (green); right: exchange of isotopes, labeling of cRGD with In-111 (blue) and cRAD with Lu-177 (green).

With dual isotope imaging, we now have a method at hand to probe the interactions of more complex nanostructures within a living being and to get a better understanding of how material parameters relate to different biodistributions.

Activatable agents for image-guided drug delivery

So far, I have shown two examples related to diagnostic imaging. Nanoparticles allow us to go one step further from diagnosis to therapeutic applications like drug delivery. To the pharmaceutical industry, nanoparticles are profoundly interesting as drug carriers. Loading nanocarriers such as liposomes with drugs changes the biodistribution of the parent drug. In case the original drug is very small (< 5 nm) excretion via the kidney is fast, but so is uptake in healthy tissue such as the heart. Taken up in healthy tissue, the drug provokes unwanted toxic side effects. Putting the drug into a bigger drug carrier instead, prohibits the uptake in normal tissue and may in fact even promote uptake into tumor tissue, as the drug carrier is trapped in the leaky vasculature of a tumor. Probably the best known example is the chemotherapeutic drug doxorubicin used in several cancer therapies. Loading this drug into liposomes leads to formation of drug nanocrystals inside the carrier, which yields a particularly high loading efficiency. These nanoparticulate drugs are now clinically used thanks to their reduced toxicity profile compared with the free drug. Although clinical studies have showed that these drug carriers lead to a higher drug concentration in the tumor, they did not significantly improve treatment efficacy. One reason might be that the drug is trapped inside the carrier and leaks out too slowly to have therapeutic effects. One option to overcome this is to design drug carriers that open up in the lesion and release their therapeutic payload within the diseased tissue. One approach is to exploit endogenous effects, like disease-specific enzymes or a local variation in the pH, to trigger an opening of the drug carrier and release of the drug. Another option is to use an external trigger that allows drug release in a previously defined region. For the latter, focused ultrasound is a perfect choice. Ultrasound waves can be applied non-invasively and penetrate soft tissue up to several centimeters. In the focus spot, energy dissipation leads to an increase in temperature depending on the particular characteristics of the ultrasound wave (Figure 6). The heat itself can already be used for therapeutic applications. At Philips, a system is currently developed in which a focused ultrasound transducer is embedded in the patient bed of an MRI scanner. The MRI allows identification of a tumor tissue, and guiding of the focal point of the ultrasound to thermally ablate the tumor tissue.

With the very same machine, it is also possible simply to warm up the tissue to temperatures slightly above body temperature with the aim of disintegrating drug carriers and releasing the drug once they enter the heated tumor tissue (Figure 6) [4]. Design of such a drug carrier is a challenge. It requires stable entrapment of the drug at body temperature within the blood, but rapid release once warmed up by a few degrees. It should circulate in the blood stream as long as possible, so that potentially all the drug can be released as long as the hyperthermia procedure continues. The drug carrier of choice is in the first instance a liposome. The liposomal membrane presents a hydrophobic approximately 4 nm thick membrane that prevents hydrophilic or charged compounds from leaking out, once they are entrapped in the inner water lumen of the liposome. The secret to achieving temperature-triggered release lies in the exact composition of the lipid bilayer that can be formed from mixtures of different lipids. These mixed lipid membranes can show different phases, like amorphous, gel or liquid crystalline phases with corresponding phase transitions at well-defined temperatures. The latter can be tuned by choosing different lipids with specific physico-chemical properties. Going through a phase transition creates transient pores in the otherwise hydrophobic barrier, allowing hydrophilic drugs and compounds to be released (Figure 6).

We are able to test drug leakage and release in vitro by measuring the kinetics of drug release by analytical techniques. We can choose different lipids to optimize the drug delivery carrier, however, we do not know how the drug release process works in vivo. We cannot quantify the drug release in the warmed up tissue, nor the leakage in the blood, in a straightforward manner. Again, thanks to their versatility nanocarriers offer an option to solve this issue. Together with the drug, we can co-encapsulate MRI contrast agents, which upon release turn on or off, depending on their nature and contrast mechanism. For example, it is possible to encapsulate a chemical exchange saturation transfer agent (CEST) and a fluorine-based MRI agent with the drug inside the liposome. The CEST agent shifts the resonance frequency of the water in the inner lumen of the liposomes to a different value. MRI now allows imaging of this second water peak to create a CEST picture. At the moment the liposome opens and releases the CEST agent, this second water peak disappears, indicating the release of the co-encapsulated drug. At the same time, we observe a fluorine signal coming up in the MRI, only observable once the agent is released with the drug. As long as the fluorine agent is encapsulated inside the liposome together with the CEST agent, interactions

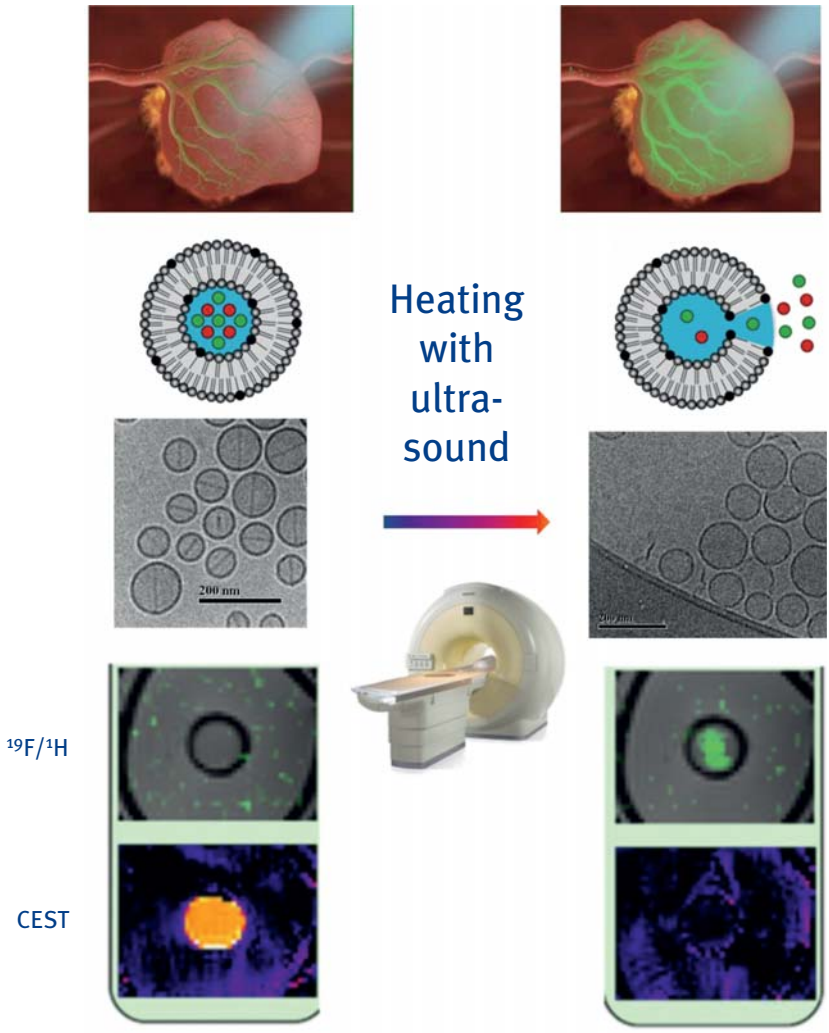


figure 6

Top row: illustration of a tumor tissue warmed up with ultrasound, showing a drug being released (green); second row: cartoon of temperature-sensitive drug carriers; third row: cryo TEM pictures of a liposome with encapsulated drug crystals and 19-F and CEST MRI agents before heating and empty liposomes after heating; fourth and fifth rows: fluorine and CEST MRI picture of a phantom filled with the above liposomal formulation. The fluorine images are overlaid on a proton image.

between these two compounds led to a line broadening of the fluorine peaks, rendering that signal undetectable (Figure 6). What did we achieve? This example shows that we can tailor nanocarriers for use in specific applications, in our case ultrasound-induced drug delivery. With this method we hope to increase and monitor the amount of drug delivered to the tumor tissue [5].

The requirements for the drug delivery system are defined by their medical application. However, it requires fundamental understanding of the underlying physics and chemistry to optimize the properties with respect to the specific application. Also in this example, the application motivated the research, but the results also increase our basic insights and understanding. The contrast observed in MRI, either in CEST images or in T_1 images, depends on the water exchange between the inner compartment and the water outside the liposome [6]. The water exchange rate is indicative for the diffusion of water molecules across the lipid membrane (Figure 7). Measuring the MRI contrast as a function of temperature allows calculation of the water exchange rate across the membrane until suddenly, around the release temperature, pores form in the lipid bilayer that promote rapid release of the payload. MR imaging and contrast agents in this case turn out to be a tool to study the transport properties of lipid bilayers and the integrity of the membrane, which has already been an academic research topic for decades.

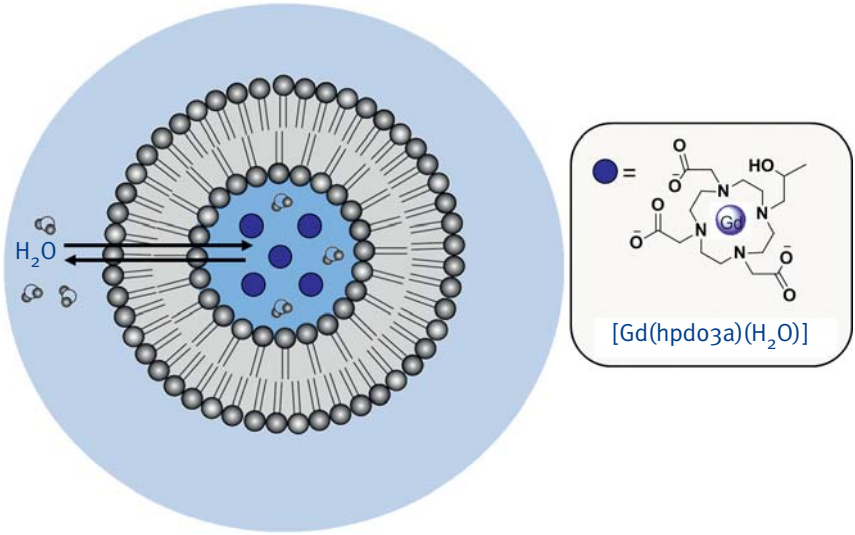


figure 7

Water exchange across the lipid bilayer of a liposome.

What's next?

Research and applications

I have shown three examples of my current research. These projects are not finished, in fact, we are just starting to recognize the tremendous complexity and many new questions are emerging. Molecular imaging is now an integral part of biological, biomedical and pharmaceutical research. It serves as a research tool to investigate mechanistic questions in biology or in drug development to quantify the drug biodistribution in vivo. In future, it will also develop towards the clinic for human application. Currently, new molecular imaging agents for nuclear imaging are in clinical trials and some will enter clinical use within the next 10 years. Most likely, these diagnostic agents will change the workflow in imaging-based diagnostics and in clinical decision-making towards a more personalized and patient-tailored treatment. So far, only two nanoparticulate diagnostic agents for intravenous injection have been approved for human use: magnetic nanoparticles as contrast agents for MRI and microbubbles for use with ultrasound imaging. The tremendous costs of clinical trials will most likely prohibit development of new nanoparticle-based diagnostic agents for economical reasons. The more profitable therapeutic market with higher financial reimbursement is sure to fuel more industrial research and development in this area. Nanoparticles are already approved in therapeutic applications as drug carriers, thanks to their enormous potential in new formulations. I am sure that in future more nanoparticles will reach clinical application. Nanoparticles that release therapeutic compounds as a reaction to the local and different environments present in diseased tissue, like disease-associated receptors or enzymes, different pH, or temperature, but that can also be visualized using diagnostic imaging. We are already working on smart peptide probes that are activated by disease-specific enzymes and are subsequently taken up into cells, providing a signal that we can use for the detection of disease markers. In another project, we are even trying to get one level deeper. Instead of looking at proteins, we are trying to promote uptake of genes into cells using ultrasound in combination with microbubbles. It is difficult to say if and when this research will move into medical application in humans, but it certainly leads to a deeper understanding of biology, and how we can intervene at a molecular level using new nanostructures.

As far as my industrial research is concerned, the assignment is clear: work towards a clinical application that in the end provides a business opportunity. For my academic research, this is not necessarily the case. The way towards an application is full of scientific questions that deserve a thorough look, asking for investigation to get to new insights. It is the curiosity that drives me, the desire to understand and to generate knowledge.

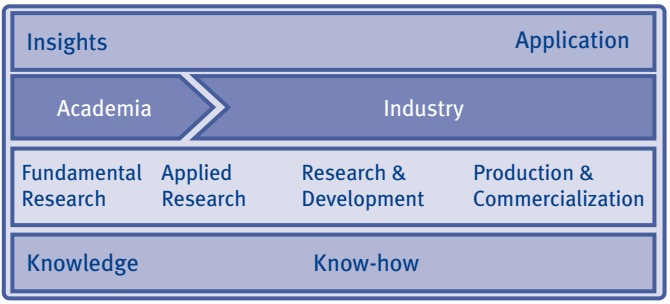
My biggest motivation, however, is the topic itself, we are working on new medical applications to diagnose and treat diseases; be it in an academic or an industrial setting. Our work is for the benefit of patients and society at large, and for me personally this aspect is a strong driver.

Academic and industrial research

From knowledge to know-how

The motivation for two of the research topics I have presented came from possible applications in the clinic to diagnose and treat diseases earlier and better. The work on spectral CT and MR-image guided drug delivery with ultrasound is a typical example in which new applications are enabled by a new technology as well as a tailored agent. The idea of working on dual isotope imaging and smart probes comes more from open questions in biology and medicine: how can we use imaging technology to study molecular interactions in vivo? Dual isotope imaging is proving to be a powerful tool for research, and we have gained more understanding of how molecules behave inside the body. I am quite sure that these insights will serve an application sooner or later.

It requires a lot of different steps to bring anything from research to a marketable product. It is not enough to have a clear idea about an application if the basic knowledge is not present, nor is it enough to have a new insight and a discovery with all required knowledge, if there is a lack of know-how about how to turn it into a marketable product. The latter is not the task of academia, while the former cannot be covered by industry. This is the reason why collaboration is useful and necessary. Applied research and industrial research laboratories play a crucial role here in bridging the gap.



As a university of technology, the Eindhoven University of Technology is well positioned to cover both fundamental and applied research. All the different disciplines ranging from chemistry, chemical biology, tissue engineering and mechanics to preclinical imaging and data analysis are present in the Department of Biomedical Engineering, providing a perfect knowledge-base for a research group on nanostructures for molecular imaging and therapy.

Industrial laboratories like Philips Research are the interface between academia and industrial research & development. I consider it the task of an industrial research laboratory to keep up with fundamental research and understand new ideas and insights as much as it is its task to understand the respective applications inside the company. Applied industrial research needs to develop basic research findings into applications. One important factor here is empirical know-how acquired by the organization over many years. This empirical know-how is preserved by individuals working on the same topic for a long time, making people the biggest asset. The trend in industry to downsize internal research is creating indirect pressure on universities to shift even more into application-oriented research. For universities, the challenge is to fulfill their primary tasks in education and basic science on the one hand, while on the other, they need to secure external funding and industrial collaborations. The latter should however never compromise the primary mission of an academic institute. I am convinced that industrial research laboratories staffed with experienced researchers are necessary and have the important function to be the ‘know-how bridge’ between knowledge and product.

Education

From contents to competence

Through my academic position, I also have the privilege of engaging in what is first and foremost the task of universities: provide adequate education. Academic research is not an aim in itself, but is intimately linked to education. Education of students has two aspects. One part is devoted to transfer of knowledge. We teach what I would call textbook knowledge: general theories and natural laws, established tools, technical skills, facts, and the currently accepted scientific paradigm of the respective fields of science. As the student advances over the years the curriculum offers more courses to specialize in different disciplines. The second aspect of education is to teach *competences*, in Dutch called 'vaardigheden'. In the beginning it is about how to apply the acquired knowledge: how to find relevant information, and how to solve more and more elaborate problems. For many, the *Master's thesis* is the final task in which both parts come together, namely applying acquired knowledge and having the competence to set-up and solve an open issue and to do independent research. Due to the broad curriculum of Biomedical Engineering, students have at the start of their Master's phase a broad knowledge base that has to rapidly deepen when going into one subject. Having a set of competences may certainly help, but being as knowledgeable as possible about facts and laws in natural science is the success factor for doing science.

In many public discussions about education, ranging from elementary school to universities, competences are often considered to be more important than knowledge. In my opinion, we should emphasize the knowledge side more. Knowledge can be fun, it may stimulate students to become curious and develop enthusiasm and passion for a field. Learning and knowledge as such can become a motivation in their own right. Teaching knowledge in general education, from school to university, is therefore important, and if it is done in a passionate way, it will stimulate the interest of students for a certain field. Also in such a broad curriculum like Biomedical Engineering, we cannot afford to compromise on teaching factual knowledge for the emphasis of competence: a solid knowledge-base is essential.

I am involved and have obligations in all aspects of student education, however, my link with industry brings with it extra responsibilities. Many of my research topics qualify as applied research, to some extent ideas and motivation come from the prospect of eventually generating economic value for a company. Yet, out of principle, I need and will always defend academic freedom of research. How do these considerations go together when I am supervising PhD projects that are in some way linked to industrial research? For me the answer lies in a more epistemic point of view. The most important point for me is to teach how to perform scientific research, how to find answers to the question of what we really know and understand, and how new experiments need to be designed to provide us with the necessary evidence to come up with meaningful conclusions and new hypotheses. We should not forget that our insights are based on experiments. The design and execution of experiments that lead to meaningful conclusions again demand a solid knowledge-base acquired through studies.

Here, the two sides of academic and industrial research come in again: we can work on the very same topic, however, it is the liberty of academia to generate knowledge, while it is the task of industrial research to turn the knowledge into know-how and eventually further into products.

The close link between academia and industrial research also presents a different setting for students offering a somewhat different learning experience. The exposure to other aspects like funding, entrepreneurship and business models while doing research provides students with an experience that can turn out to be very valuable for their later careers, whether they are in science or in industry. When talking to students I realize that many of them find it motivating to work on a topic that eventually finds an application in medical care for the benefit of patients. Collaborating as a student with a company that is driving our knowledge on molecular imaging and new therapies towards the market can be very stimulating, and can open new possibilities – in the end, there is ample know-how in a company to benefit from.

I see the passion and devotion my students have for their topics and enjoy this every day.

Acknowledgment

Persons that made this day happen

First of all, I would like to thank the board of the Eindhoven University of Technology and my colleagues of the Department of Biomedical Engineering for their trust and support that allows me to set up my research group at the University. I especially would like to thank Professor Klaas Nicolay for his support and collaboration to establish my research within his Biomedical NMR group. Dear Klaas, thank you very much for all the stimulating discussions and all the interesting projects we have started together over the last years. I am very much looking forward to the next years when many of our ideas will flourish and generate new insights.

Looking back, there have been many people who have had a great impact on my (scientific) career and in one way or another have contributed to this special day. I feel very sorry that I can't mention all of them today, but I would like to express my gratitude to a few people to whom I owe a lot.

Meinem Doktorvater Herrn Professor Woermann, verdanke ich meine wissenschaftliche Ausbildung und viele Ansichten und Prinzipien, denen ich versuche treu zu bleiben. Lieber Herr Professor Woermann, ich werde Sie immer für zwei Zitate in Erinnerung behalten, die meine wissenschaftliche Laufbahn und Denken beeinflusst haben: „Gute Meßpunkte haben Ewigkeitswert“ und „Ich bin der letzte Dinosaurier“. Das erste Zitat hat mich immer inspiriert und erinnert, dass *knowledge* letztlich immer auf experimentellen Resultaten basiert und diese mit größtmöglicher Gewissenhaftigkeit erzielt werden müssen. Das zweite Zitat reflektiert die Einsicht, dass sich die Forschungsumgebung stark verändert hat und sich weiterhin verändern wird. Die Frage ist immer, wie weit man sich anpassen kann und will. Ihr Idealismus, der Drang Dinge zu verstehen und das kompromisslose Festhalten an der Grundlagenforschung hat mich immer inspiriert und ich hoffe ein paar dieser Qualitäten und Einsichten an die folgende Generationen weitergeben zu können. Vielen herzlichen Dank, Herr Professor Woerman.

Dear prof. Rachel Yerushalmi-Rozen, I spent wonderful years with you in Israel. Rachel, I was not sure if I should refer to you as a colleague, friend or family, as you are all of these to me. You taught me a completely different look at science and life: out of the box, creative but pragmatic, dealing with the problems of today rather than the worries of tomorrow. Although I don't see you that often: אפשר להיות רחוק ולהרגיש קרוב

Dear prof. Alan Esker, I will always remember our time at NIST, where we spent days and nights at NG7 doing really great science and discussing politics. Thanks a lot for coming over today.

At Philips Research, I would like to thank Henk van Houten, Hans Hofstraat, Franklin Schuling and Oliver Steinbach, who are responsible for the molecular medicine related research program and who have given me all their support over the last years, and who have also supported my part-time assignment at the Eindhoven University of Technology.

In 2004, we started out with a handful of scientists into this endeavor of molecular imaging and therapeutic research. This small team sowed the seeds of many of the activities I have addressed today, and grew into a whole group. Dear colleagues, I am very grateful for all your spirit and efforts to bring this field towards a medical application in the clinic, and for all the fantastic ideas and discussions we have had and still have. Thank you for that.

I owe much of my motivation and enthusiasm to a very special group: my PhD students. What a professor should mean to his PhD students has been discussed many times, but far fewer words have been spent on what PhD students mean for a professor. I feel honored that you joined me, but most important to me is to see how you get into science, how your curiosity increases with every open question, how your passion increases with the results, and how you grow along the way into independent scientists. Observing your enthusiasm for your research is very rewarding to me, and that is why I also love my work at the University so much. Anke, Sander, Mariska, Pedro, Nicole and Tiemen, van harte bedankt.

Finally and most importantly, my family: Liebe Eltern, Ihr habt mich jahrelang unterstützt in meinem lang gehegten Jugendtraum, eines Tages in die akademische Forschung zu gehen. Der Weg zu dem heutigen Tag hatte viele Umwege, und daß aus meinem Traum nach mehr als 25 Jahren letztlich doch Realität geworden ist, verdanke ich auch Euch, wofür ich Euch herzlich danken möchte.

Above all, I would like to thank one special person, with whom I have now shared my life for more than 20 years, who has accompanied me through all my studies, who went with me abroad to the US, to Israel, to settle with me in the Netherlands. Kerstin, without your love and support I wouldn't be here today. I know that I sometimes ask a lot from you, you had to restart your career each time we moved on, making many compromises. I owe you this day and much more in my life. You and our lovely daughter Yaël, you both are the most precious part of my life.

Ik heb gezegd.

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Curriculum Vitae

Prof.dr. Holger Grüll was appointed part-time professor in Nanoconstructs for Molecular Imaging and Therapy at the Department of Biomedical Engineering of the Eindhoven University of Technology (TU/e) on 1 May 2007.

Holger Grüll (1968) graduated in chemistry (1993) and gained his PhD in Physical Chemistry from the University of Cologne. After his PhD, he went as a visiting scientist to the Ben-Gurion University of the Negev, Beer Sheva, Israel. From Israel, he moved to the National Institute of Standards and Technology in Gaithersburg (USA) on a fellowship from the Alexander von Humboldt Society (1997-1999), working on polymer thin films and nanoparticles. After his stay in the US, he returned for another year to the Ben-Gurion University. In 2000 he started his career at the Philips Research Laboratory in Eindhoven on sensor technologies for in-vitro diagnostics. Since 2004, he has been responsible at Philips for research on agents for molecular imaging and therapeutic applications. He was appointed part-time professor at TU/e in May 2007. His chair focuses on nanostructures for use in molecular imaging and therapy.

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