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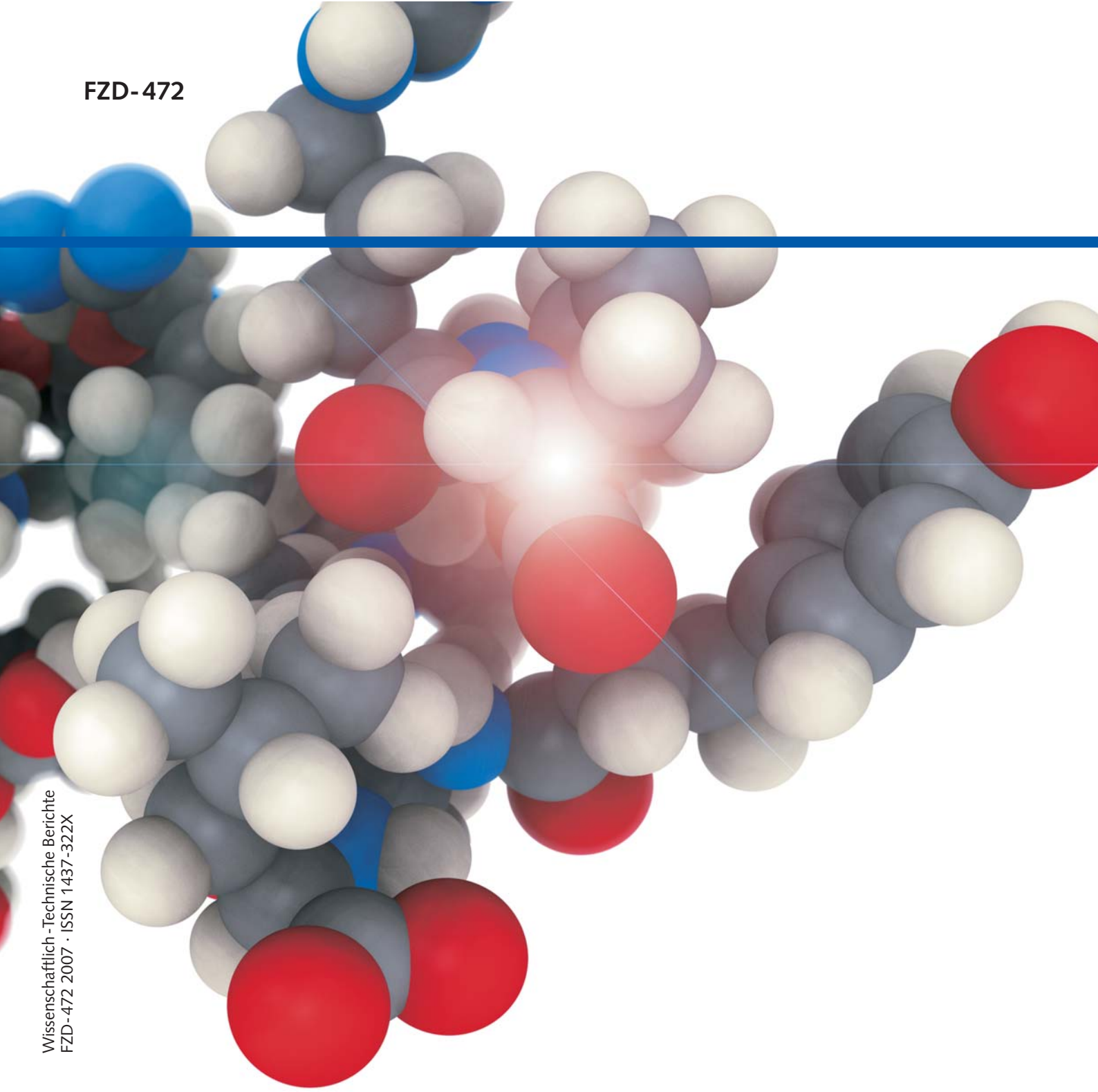

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LIFE SCIENCES

TRIENNIAL SCIENTIFIC REPORT 2004 -2007 | Volume 3

Forschungszentrum
Dresden Rossendorf

Content

Cover picture: 3D scheme of the molecular structure of a multimeric neurotensin derivative (Holger Stephan, graphic: Sander Münster)

Preface

Roland Sauerbrey I Scientific Director

This volume of the Triennial Scientific Report highlights the scientific output of the FZD research program "Life Sciences", covering the years 2004 to 2006 and the first six months of 2007. It is one out of three volumes that are published this year for the first time. The first part of this report introduces the "Life Sciences" program as well as the large-scale facilities that are used for research within this program. The second part consists of ten articles on research projects that were conducted by scientists of the Institute of Radiopharmacy and the Institute of Radiation Physics addressing current research into cancer-related biomolecular function, imaging, and therapy.

The last eighteen months were characterized by an intense discussion about the future of the Forschungszentrum Dresden-Rossendorf (FZD). In meetings and seminars we debated about our topical status and our future, asking questions like: "What are our future scientific objectives?" "Which research methods and facilities are required in order to reach those goals?" "How can new research activities be funded and who are our future cooperation partners?"

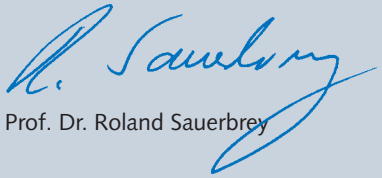
As for the Life Sciences research program scientists at OncoRay - Center for Radiation Research in Oncology - have become the most important cooperation partners. The FZD is one out of three members of this German Federal Ministry of Education and Research (BMBF) funded "Center for Innovation Competence". It is actively engaged in promoting an ion-therapy center in Dresden, which would present a very promising location given the scientific background of the OncoRay center that is supported by the University Hospital Dresden and the Technische Universität Dresden on the one hand and the expertise of FZD scientists on the other hand. For example, scientists from the FZD Institute of Radiation Physics have developed an effective system of online-monitoring for the ion-beam radiotherapy facility at the Gesellschaft für Schwerionenforschung (GSI) in Darmstadt. Another example: the Institute of Radiopharmacy performs tumor research on small animal models in close collaboration with OncoRay using a variety of tools and methods at the molecular-imaging center for small animals of the FZD.

The newly established collaboration of the OncoRay center with the ultraoptics center in Jena, which started at the beginning of 2007, promises new impulses for radiotherapy to combat cancer in the future. This exciting "onCOOPtics" project, also funded by the BMBF, with laser physicists from Jena, radiotherapy experts from the University Hospital Dresden, and scientists from the FZD, is dedicated to the fundamental understanding of high-energy laser processes for acceleration of ions, which is required to explore their yet

unexploited potential for novel therapeutic approaches in the next decade. A high-intensity laser laboratory is currently under construction at the FZD and will be put into operation in the beginning of 2008. The Laser-Particle Acceleration Group that is responsible for this laser was founded in November 2006 and comprises six scientists and laser engineers now. All these steps undertaken by us and our new cooperation partners in Dresden and Jena are building blocks of our common vision to turn Dresden into an excellent tumor research location designed to produce first-class scientific results in its own right.

The new high-power laser facility at the FZD will at the same time strengthen the interdisciplinary cooperation of scientists from different institutes of the FZD. For example, the Institute of Radiation Physics will investigate the effect of particle radiation produced by high-intensity lasers on living cells. Ph.D. work on dosimetry with respect to future radiotherapy using high-intensity lasers has already started under the supervision of scientists from OncoRay and the FZD. Scientific groups of the Institute of Ion-Beam Physics and Materials Research as well as the Institute of Radiation Physics are interested in basic questions of particle-matter interaction. Alternative concepts for the acceleration of particles like plasma wakefield will be the common focus of physicists from the Radiation Source ELBE and the Laser-Particle Acceleration Group. Especially laser driven wakefield for the post-acceleration of ELBE electron bunches are to be mentioned here.

Finally, I would like to thank our partners in both the state and the federal government for their continued support, our national and international scientific cooperation partners for many successful joint research endeavours and, last but not least, the entire staff of the FZD for their multidisciplinary contributions to the improvement of human health using state-of-the-art physical approaches.



Prof. Dr. Roland Sauerbrey

Life Sciences program

at the Forschungszentrum Dresden-Rossendorf

Jörg Steinbach

The scientific activities in the Life Sciences research program focus on molecular approaches to tumor diagnostics and therapy. This is motivated by the fact that in Germany, malignant tumors account for 25% of mortality. To be more specific, every 6th person between the ages of 20 and 65 years dies from cancer. Today, cancer is treated with three fundamental therapies: surgery, radiation therapy, and chemotherapy. Curative treatment, however, fails for about half of tumor patients. Radiation therapy may be improved considerably by combining technologically-advanced external radiotherapy, radionuclide therapy, and molecular targeting methods. The Life Sciences research program focuses on investigations related to the disease-relevant behavior of cells and tissues as well as biomolecular structure and dynamics using *in vitro* studies, *in vivo* experiments, and clinical studies in patients. Additional research aims to improve diagnostics and therapy.
The Institutes of Radiopharmacy and Radiation Physics contribute to this research program. They bring together expertise from radiopharmaceutical science, radiation physics, and spectroscopy using a unique combination of resources, such as the Radiation Source ELBE and the PET facility in combination with Radiopharmaceutical Chemistry and Radiopharmaceutical Biology at the FZD.

(i) Radiopharmaceutical tumor research

A detailed understanding of cancer-related molecular and cellular processes is required to develop new methods for sensitive and specific diagnostics and therapy. Therefore, a major goal is to improve Molecular Imaging and Therapy of Tumors complemented by metabolic research based on radiopharmaceutical methods. In so doing, we aim to identify new biomolecules which may be used as targets for radioactively-labeled tracers in

Photo: Jürgen Lösel

diagnostics or therapy of solid tumors, metastases, or inflammatory processes. Eventually, we will develop new targeting concepts for drugs labeled with particle-emitting radionuclides. Their radiation is capable of destroying tumors, but spares healthy tissue. Also, we are investigating diagnostically and therapeutically relevant radionuclides, in particular particle emitters.

Preclinical studies for pharmacological characterization of appropriate substances and predrugs are part of these research activities. The studies include different kinds of molecular imaging in animal models of disease in order to investigate processes *in vivo* and to enable the characterization and preclinical application of newly developed substances. Yet, molecular imaging generates large amounts of data. Data handling and image correction, i.e., for patient movement, are therefore both important parts of our work. In order to translate our research results to a clinical environment, the FZD and the Technische Universität Dresden are collaborating in a common PET center located on the grounds of the FZD.

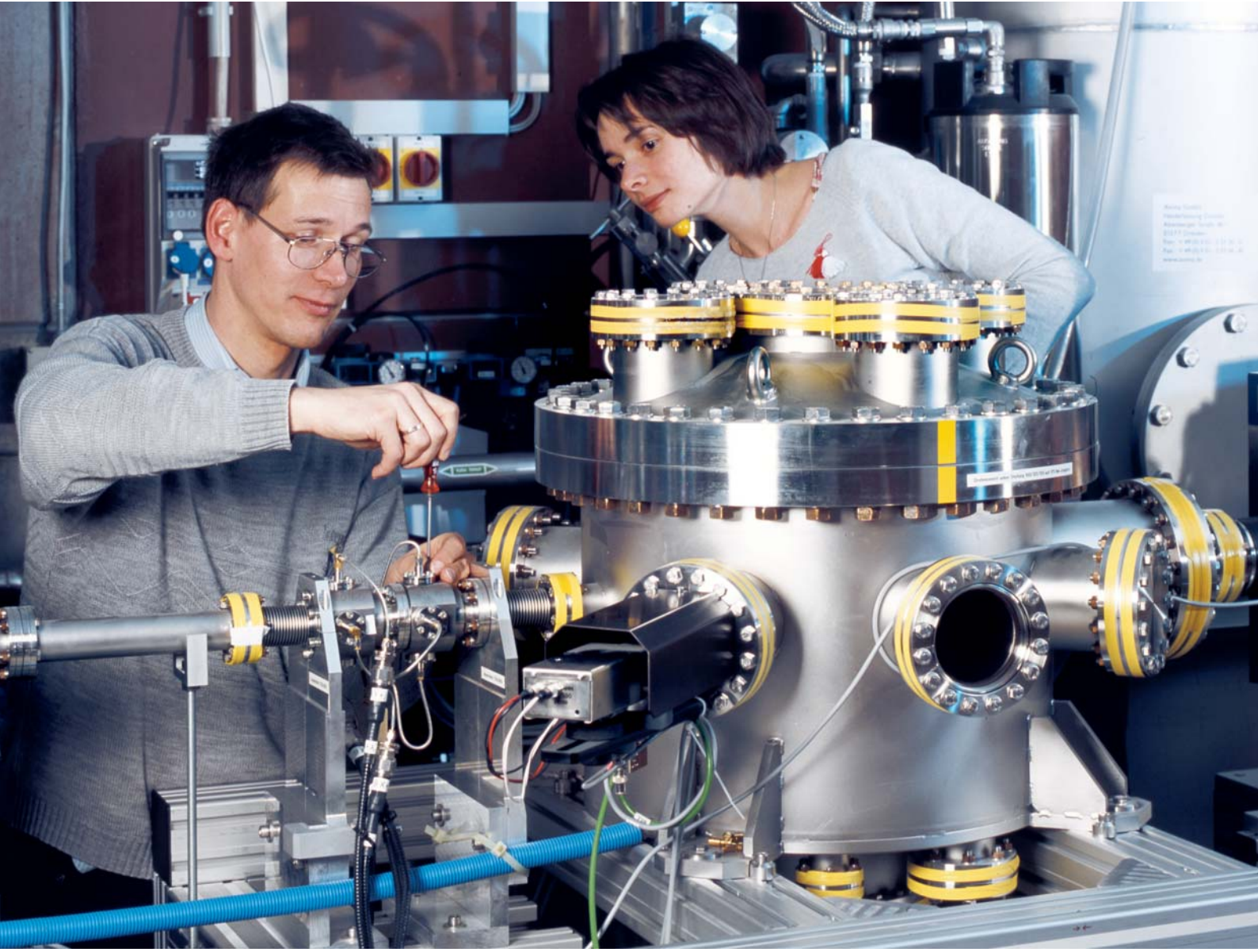
(ii) Radiotherapy monitoring and radiation damage

Today, more than 50% of tumor patients receive radiation treatment—and that number is rising. The crucial challenge of radiotherapy is to destroy the tumor completely while saving the surrounding healthy tissue. Yet, in many cases radiotherapy, which is based on photon or electron beams delivered by compact electron linear accelerators, fails. Therefore, new technologies for generating and monitoring radiotherapy beams need to be developed and transferred to clinical application, which is the subject of our research. Due to their favorable physical and radiobiological properties, ion beams have recently started being used in radiotherapy. A unique method for image-guided radiotherapy, in-beam PET, is applied to clinical ion beam treatments. We are also investigating whether other kinds of radiation like ultra-hard photons can be applied to cancer treatment. Moreover, the radiobiological properties of new radiation qualities which are interesting for medical application are tested by *in vitro* experiments with both tumor and normal tissue cells. Currently, the main experimental facility used for these experiments is the ELBE radiation source delivering novel unconventional beams, such as very intense ultra-short pulsed electron beams and monochromatic tunable X-rays. In the future, we also plan to investigate laser-accelerated particle beams with respect to their potential for cancer treatment.

(iii) Dynamics of membrane receptor structure

Structural transitions within biopolymers, such as DNA and proteins, play key roles in many physiological disorders including cancer. Detailed knowledge of these molecular switching processes is particularly required for membrane proteins. Here, structural information is very limited and especially desired for G-protein-coupled receptors (GPCRs). They are targeted by 50% of the current pharmacotherapeutics and are involved in the genesis of various cancerous diseases. Using infrared spectroscopy, functionally-relevant structural transitions are studied in real time to systematically understand these molecular switching mechanisms in membrane proteins. New strategies for GPCR-based diagnostics and therapies are being explored in collaboration with industrial partners.

All these activities are primarily embedded in a scientific network with the Faculty of Medicine and the Faculty of Mathematics and Natural Sciences of Technische Universität Dresden. The scientific program is coordinated in close cooperation with the Center of Radiation Research in Oncology (OncoRay®) which is jointly operated by Technische Universität Dresden, FZD, and the University Hospital Carl Gustav Carus Dresden and hosted at the Faculty of Medicine of TU Dresden. We are also a member of the Center for Regenerative Therapies Dresden which is increasingly important for our future scientific cooperation.



Jörg Pawelke and Anna Lehnert at the X-ray beamline at ELBE.

Facilities for Europe

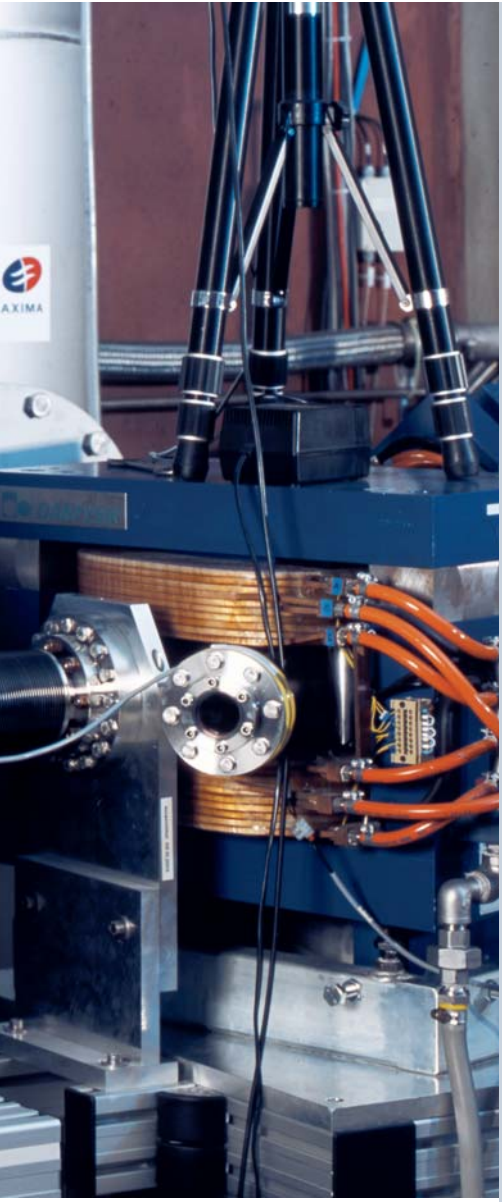
The Radiation Source ELBE

Jörg Pawelke, Karim Fahmy

The basic component of the Radiation Source ELBE (Electron Linear accelerator with high Brilliance and low Emittance) is a superconducting electron linear accelerator which provides an electron beam with an average current of up to 1 mA and beam energy of between 5 and 40 MeV. The particular properties of this primary beam are its low transverse emittance (i.e., a nearly parallel beam of small spot size), short (~ps) pulses, low energy spread, and flexible temporal structure. These outstanding properties allow a variety of secondary radiations for experiments in basic as well as applied research. In the Life Sciences program, experiments are focused on the Biostructures and Radiation research area utilizing (i) intense infrared light beams delivered by a free-electron laser (FEL) for biophysics research and (ii) novel unconventional beams of ionizing radiation which are of potential medical interest for tumor diagnostics and therapy.

Monitoring and manipulating biomolecular structures by FEL infrared light

The high IR-pulse repetition (13 MHz) and, correspondingly, the high energy which is emitted with low divergence from the ELBE-FEL allow biophysical studies where these parameters are critical. For example, it has been shown that the high energy of the ELBE-FEL even enables the chemical characterization of single layers of biomolecules on reflecting surfaces using IR reflection absorption spectroscopy. Due to the narrow spot size of the IR beam and its well-defined incident angle, such measurements can be performed with a spatial resolution close to the diffraction limit. In combination with the intrinsically high time-resolution of the FEL, this will allow sensitive experiments on fast structural transitions in monomolecular layers on solid supports, as they are applied in many areas of biotechnology. In addition, it has been shown that disease-related biomolecules, such as DNA, can be structurally manipulated using FEL light (Fig. 1). Current research utilizes various FEL-pulse regimes to generate layers of DNA with spatially defined structural alterations which are of great interest for biotechnological applications.



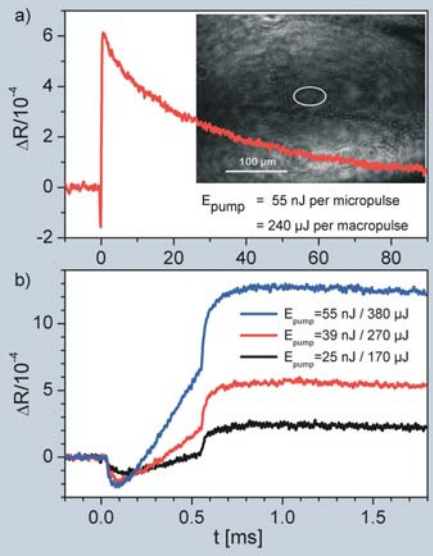


Fig. 1: The Brewster-angle-microscopic image of a DNA film of 1 µm thickness. The central area of 40 µm diameter was exposed to IR-FEL macropulses at a wavelength of 9 µm which are absorbed by the chemical groups of the DNA backbone. Structural transitions induced by picosecond micropulses at a repetition rate of 13 MHz accumulate during the 400 µs long macropulse causing an almost linear increase in reflectivity (bottom). After exposure to the macropulse, the relaxation of the DNA structure proceeds on a ms time scale (top).

New radiation qualities for radiooncology

In some cases, state of the art radiotherapy fails to destroy the tumor completely while saving the surrounding healthy tissue. This requires new technologies in generating, forming, and monitoring radiotherapy beams which are developed with the help of experiments at ELBE. For this purpose, new radiation qualities are utilized. Their radiobiological properties must be investigated for both tumor and normal tissue cells. Both the new radiation qualities and ELBE experiments have proven advantageous in several ways: (i) beams of ultra-hard photons can potentially be better focused on the tumor; they are monitored by the in-beam PET method at ELBE. (ii) The high-current electron beam of ELBE allows unique experiments of split-dose cell irradiation. This means that a priming radiation exposure is followed by re-irradiation after a delay lasting for any desired time period from milliseconds up to a few minutes. Priming radiation exposure is followed by the early steps of DNA damage recognition and repair, which brings about enhanced cell killing after re-irradiation. But the radiobiological consequences of short pulse irradiation can also be investigated. This is interesting for future compact particle accelerators which are developed on the basis of high-intensity laser systems. Such an accelerator will deliver ultra-short and intense bunches of particles comparable with those of the ELBE electron beam. (iii) The low emittance of the electron beam allows generating channeling radiation, i.e., monochromatic X-rays, which is tunable in the 10 to 100 keV range (Fig. 2). X-rays in this energy range are widely used in medical diagnostics, and experiments at ELBE can provide photon-energy resolved information on basic radiobiological effects and mechanisms following an exposure of living cells to X-rays.

Fig. 2: Beamline of the channeling X-ray facility at ELBE. The electron beam coming from the ELBE accelerator (from the left) and entering the vacuum chamber (in the middle) hits a single diamond crystal. Channeling radiation is emitted by the relativistic electrons when traveling through the crystal along a lattice plane.

Chemistry student Katrin Müller in a radiopharmaceutical laboratory.

The PET Center

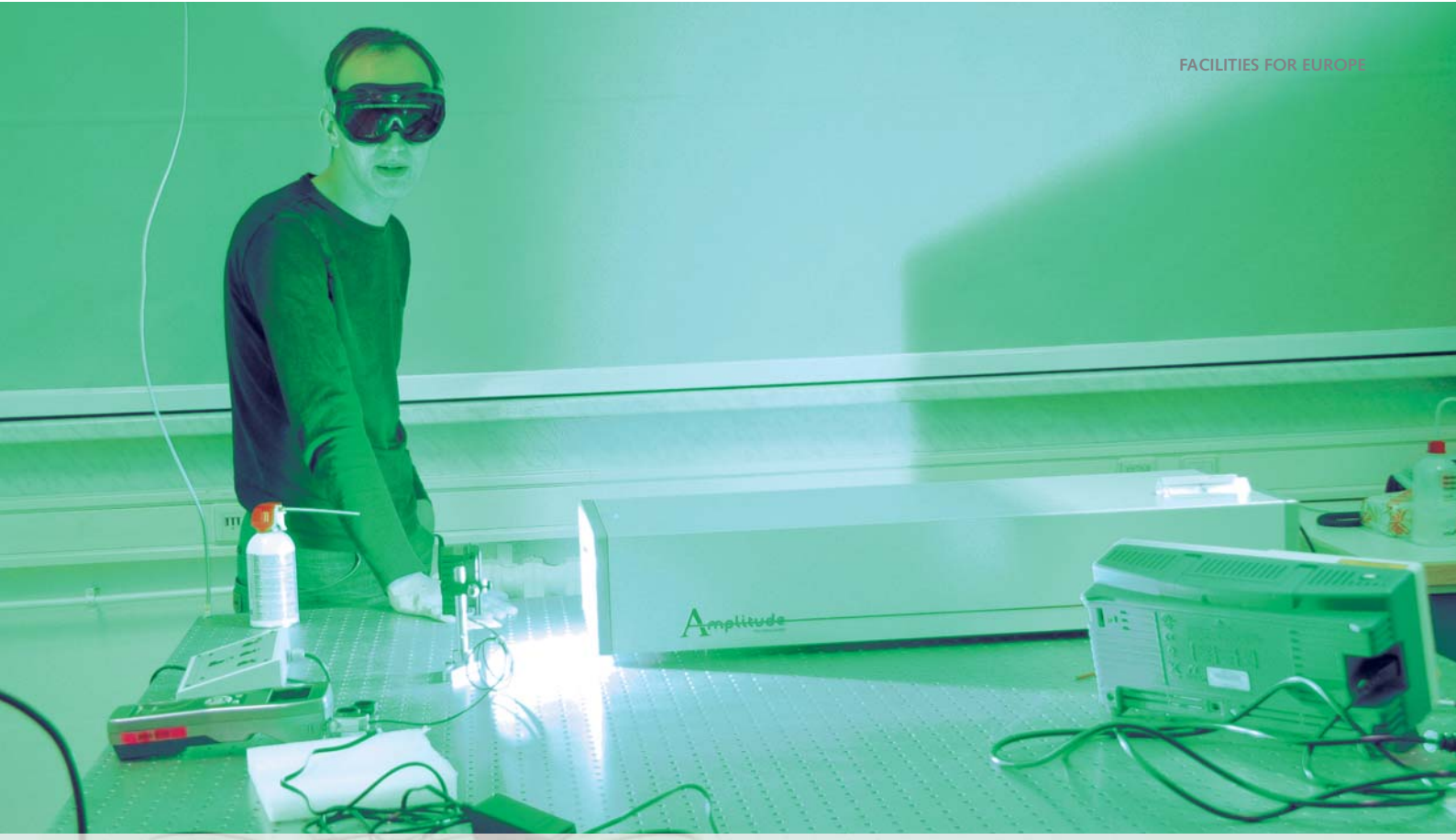
Jörg van den Hoff

The Positron Emission Tomography (PET) Center is jointly operated by the FZD and the Technische Universität Dresden. The infrastructure comprises a dedicated cyclotron, GMP (Good Manufacturing Practice) approved radiopharmaceutical laboratories, and several tomographs: a human PET tomograph and, for small laboratory animals, dedicated tomographs for PET, magnetic resonance imaging and spectroscopy (7 Tesla MRI/MRS), and computed tomography (CT).

As its central resource, the PET Center provides molecular imaging capabilities for noninvasive in vivo investigation of physiological and biochemical processes related to different pathophysiological states, notably in tumor diseases. The PET technique is able to noninvasively deliver quantitative information on cellular transport processes and metabolism (tissue perfusion, distribution volumes, turnover rates, and so on) in an unparalleled way. PET utilizes radioactive tracers at the nano- and picomolar concentration level, thus excluding any interference of the measurement with the systemic metabolism. PET accurately provides the three-dimensional distribution of the tracer concentration with a spatial resolution of better than 2 mm in the case of dedicated small animal tomographs. Performing these measurements allows assessing the time dependence of the tracer distribution and, thus, quantification of relevant pharmacological parameters.

Research at the PET Center is focused on investigations of transport, metabolism and signal transduction in normal tissue as well as damaged and tumor tissues with the PET method. Our multi-disciplinary research group of radiochemists, biochemists, biologists, physicists, software engineers, and physicians addresses this task by the development of new PET tracers, biological characterization of their properties, multi-modal imaging of small animal tumor models with new and established tracers, development of new image data acquisition and evaluation techniques, and, finally, clinical studies in humans.

An important part of this work is to advance biologically individualized, technically optimized radiotherapy, which is also the main goal of the Center for Radiation Research in Oncology "OncoRay" recently founded in Dresden (the FZD being a founding member). In this context, our efforts are focused on the systematic evaluation of various tracers for assessing tumor vitality and radiation sensitivity and the subsequent seamless integration of the quantitative information derived from PET into radiation treatment planning and therapy response control. Secondly, research at the PET Center focuses on the investigation of pathomechanisms of metabolic and inflammatory diseases such as the metabolic syndrome. Apart from these research projects, our imaging techniques are also utilized in pre-phase I clinical trials for drug development in collaboration with the pharmaceutical industry.



Stefan Bock preparing laser experiments.

Laser-Particle Acceleration (Laboratory)

Ulrich Schramm

Over the past several decades, particle accelerators have developed into versatile tools for many aspects of science. They provide beams for such diverse areas as basic research, brilliant light sources, as well as diagnosis and therapy in life sciences. However, acceleration and especially transport of energetic particle beams requires large installations, which has always fostered great interest in novel techniques which could circumvent these restrictions. Within just the last few years, laser-particle acceleration—a technique which uses relativistic laser plasma interaction to provide accelerating fields which exceed conventional ones by at least three orders of magnitude—has matured in a way that allows us to envision the first applications. High-energy (GeV) electron beams of only a few millimeters as well as intense high-quality ion bunches have been generated.

Therefore, FZD decided to establish a high-power laser laboratory planned to deliver the first 100 Terawatt laser pulses to targets by early 2008. The laboratory has been installed in close proximity to the ELBE accelerator in order to bundle the expertise in state-of-the-art superconducting accelerator and gun and the laser accelerator technology.

The most ambitious goal of this group is to develop a compact and reliable laser-ion accelerator which can be positioned close to an irradiated object, thus eliminating the need of costly ion beam lines. Development of dedicated laser targets as well as of high-power laser technology will be performed in-house. Within the framework of onCOOPtics—a cooperation project between laser experts from Jena and oncologists from Dresden funded by the Federal Ministry of Education and Research (BMBF)—irradiation of tissue probes with laser accelerated particles will be studied so that at last a compact machine for cancer therapy could be envisioned.

In addition to the scattering of laser photons from ELBE, accelerated electrons will be exploited as a variable and fully synchronized source of soft X-ray radiation. Unprecedented photon yield can be expected from combining the ultra-brilliant ELBE photo-gun, which is presently being developed with a laser beam of a matched repetition rate. A source like this will pave the way for time-resolved X-ray imaging and future diagnosis techniques, such as phase contrast imaging. It will be developed in collaboration with the Fraunhofer Institute for Applied Optics and Precision Engineering (IOF) in Jena.

Research

PET-tracer development: from basic research to clinical applications

Cathleen Haase, Ralf Bergmann, Jens Pietzsch

In recent years, positron-emission tomography (PET) has evolved as a valuable imaging modality in oncology, neurology, cardiology, rheumatology, and other medical branches. In oncology, for instance, PET has become an essential tool for displaying cancer, monitoring treatment response, investigating tumor recurrence, and for objective assessment of overall therapeutic efficiency. Apart from these clinical applications, PET plays an ever-increasing role in basic and applied tumor research due to its inherent property of depicturing and functional characterization of physiologic, metabolic, and molecular pathways in the living organism in a quantitative manner. Several radiopharmaceutical compounds have been developed for PET which have proven suitable for imaging and functional characterization of tumors. In this regard, amino acid-based PET tracers are especially important. It is well known that the uptake of amino acids is increased in tumor tissue compared to normal "healthy" tissue. Among various available amino acid-based radiopharmaceuticals, the fluorine-18-labeled amino acid "3-O-methyl-[^{18}F]fluoro-L-DOPA" ([^{18}F]OMFD; Fig. 1), which has been developed and evaluated by the Institute of Radiopharmacy at FZD, is a very promising PET tracer and has already been successfully applied in brain tumor imaging in close cooperation with the Clinic and Policlinic of Nuclear Medicine at the University Hospital Carl Gustav Carus Dresden (Fig. 2) [1, 2].

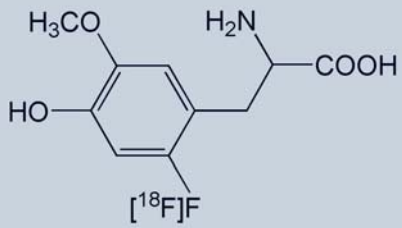


Fig. 1: Chemical structure of the fluorine-18-labeled amino acid derivative 3-*O*-methyl-6-[^{18}F]fluoro-L-DOPA ([^{18}F]OMFD).

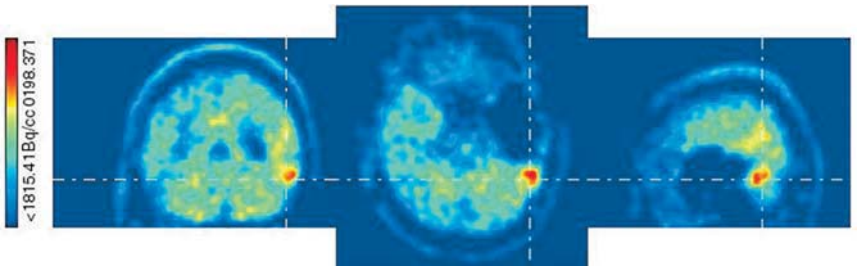


Fig. 2: Positron emission tomography (frontal, transversal and sagittal projection) of the brain with [^{18}F]OMFD: brain tumor recurrent (astrocytoma) 4 years after surgical resection and postoperative radiotherapy. The figure shows the distribution of ^{18}F radioactivity, the red color indicating the highest radioactivity.

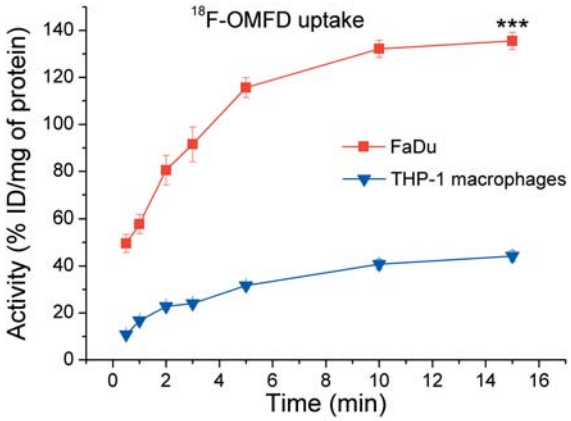


Fig.3: Time course of [^{18}F]OMFD (2 MBq/mL) uptake into tumor FaDu cells and THP-1 macrophages. *$P < 0.05$.

The aim of our current research is to determine whether [^{18}F]OMFD also has potential in the diagnosis of clinically relevant tumor entities other than brain tumors. Moreover, we evaluated the potential of [^{18}F]OMFD for differentiation of tumorigenic and inflammatory processes, which is an important challenge in nuclear medicine. Furthermore, we focus on cellular and molecular characterization of different tumor entities *in vitro* and *in vivo,* thereby gaining new insights into the mechanisms of proliferation and gene expression. We investigated the main amino acid transport systems for the uptake of [^{18}F]OMFD in various cells, e.g., in human head and neck squamous cell carcinoma cells (FaDu) and in the phorbol ester stimulated human monocyte/macrophage cell line (THP-1). We also studied [^{18}F]OMFD uptake in the corresponding tumor (FaDu) xenograft models in nude mice *in vivo*. For molecular

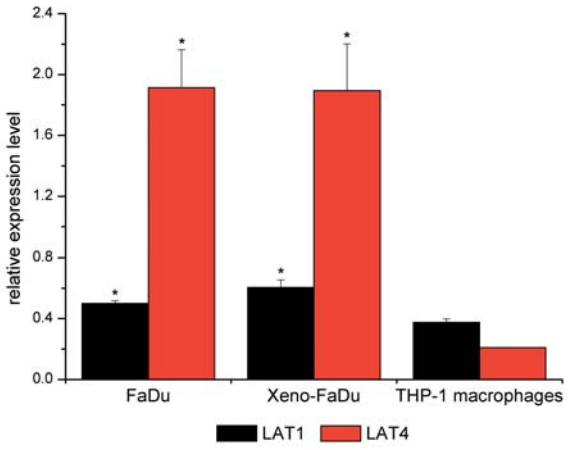


Fig. 4: LAT1 and LAT4 gene expression studies in FaDu tumor cells and the corresponding xenografts, and THP-1 macrophages using quantitative real-time Polymerase Chain Reaction. Arithmetic chart of the threshold cycle (C_t) for LAT1 and LAT4 was normalized to house-keeping-gene 18S-rRNA expression. $^*P < 0.05$.

characterization of the amino acid transport systems, detailed quantitative gene expression analyses of the different transporter subtypes within the so called L-amino acid system (L-system; LAT1, LAT2, LAT3, LAT4 and 4F2hc subtype) and the alanine-serine-cysteine system (ASC system; ASC1 and ASC2) were performed.

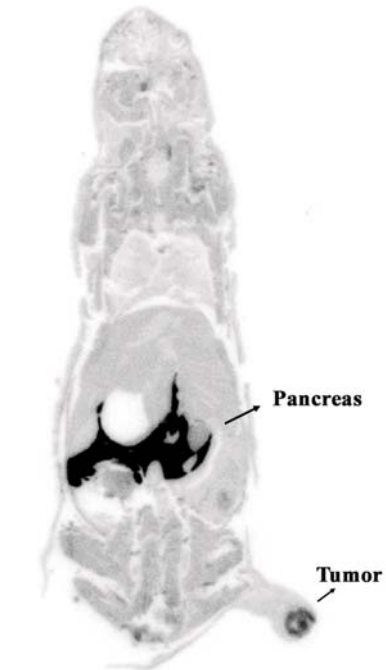


Fig. 5: Representative small animal positron emission tomography image (maximum intensity projection) of a mouse human-head and neck squamous cell carcinoma xenograft model (FaDu) after intravenous administration of 10 MBq of [^{18}F]OMFD (60 min p.i.). The highest radioactivity concentration can be observed in the tumor and in the pancreas. The latter is of particular interest and is hypothesized to indicate high amino acid transport activity in this organ due to increased amino acid requirement for the synthesis of several hormones and enzymes.

It was demonstrated that the uptake of [^{18}F]OMFD in all cell lines tested was mediated mainly by the sodium-independent high-capacity L-system. Kinetic analyses in cells demonstrated an increased uptake in the human head and neck squamous cell carcinoma (FaDu) cells in comparison to the THP-1 cells (Fig. 3). The latter were used as a model for tumor-associated (proinflammatory) macrophages. The accumulated radioactivity in the FaDu cells was three times higher than in THP-1 cells (Fig. 3). The relative expression level of the L-amino acid transporter subtypes LAT1 and LAT4 in FaDu tumor cells and the corresponding FaDu xenografts was significantly higher when compared to THP-1 macrophages (Fig. 4) [3]. These data gave rise to the assumption that [^{18}F]OMFD is not only a good tracer for brain tumors but also for other, especially poorly-differentiated tumor entities, e.g., head and neck squamous cell carcinoma. To substantiate this assumption, small animal PET studies were performed in tumor-bearing mice xenotransplanted with human head and neck carcinoma cells (FaDu), also revealing a high [^{18}F]OMFD uptake in tumor tissue (Fig. 5) [3].

In conclusion, an important finding is that [^{18}F]OMFD offers the opportunity to study the specific L-amino acid transport system in certain tumor entities overexpressing this transport system with PET *in vivo*. The use of [^{18}F]OMFD may allow quantification of important cellular processes related to tumor proliferation in these tumor entities. However, the contribution of endothelial cells and proinflammatory cells like macrophages and the role of different LAT subtypes on the overall uptake of [^{18}F]OMFD in tumors and inflammatory lesions must be researched further.

References*

[1] *Radiotherapy Treatment Planning in Brain Tumors with ^{18}F-3-O-methyl-fluordopa (^{18}F-OMFD) and Positron Emission Tomography (PET)*, H. Alheit[1], L. Oehme[1], C. Winkler[1], F. Füchtner, A. Hoepping[2], J. Kotzerke[1], B. Beuthien-Baumann[1], European Journal of Nuclear Medicine and Molecular Imaging (2007), submitted

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Project partners

[1]Clinic of Nuclear Medicine, University Hospital Carl Gustav Carus Dresden, Germany

[2]ABX Advanced Biochemical Compounds GmbH, Radeberg, Germany

*In this report we quote mainly the most important papers that were published by FZD scientists and their partners.

Small animal positron-emission tomography in radiation therapy response monitoring

Ralf Bergmann at the MICRO-PET device of the FZD.

Ralf Bergmann, Jens Pietzsch, Bettina Beuthien-Baumann[1,2]

Tumors are abnormal masses of tissue that result from unusually high cell division or retarded cell death. A fundamental property of neoplasia is the Warburg effect, which means that tumors have a high metabolic rate and accumulate glucose at a higher rate than normal tissue. 2-[^{18}F]fluoro-2-deoxy-D-glucose ([^{18}F]FDG) Positron-Emission Tomography (PET) assesses this tumor property. [^{18}F]FDG is an analog of deoxyglucose that has been employed for tumor imaging using the radioactive label ^{18}F (110 min half-life) (Fig. 1). The molecular [^{18}F]FDG-PET imaging technique offers a complementary approach to anatomic imaging, such as computer tomography and magnetic resonance imaging, and is more sensitive and specific in detecting certain cancers. [^{18}F]FDG-PET has been widely applied in oncology, primarily as a staging and restaging tool that can guide patient care. But as it accurately detects recurrent or residual diseases, [^{18}F]FDG-PET also has significant potential for assessing therapy response. In this regard, it can improve patient management by identifying responders at an early stage before the tumor size is reduced; in contrast, nonresponders could discontinue futile therapy. Moreover, the reduction of the [^{18}F]FDG-PET signal within days or weeks after initiating therapy (e.g., in lymphoma, non-small cell lung, and esophageal cancer) significantly correlates with prolonged survival and other clinical endpoints. Applications of [^{18}F]FDG include assessing response to therapy also for tumor models. [^{18}F]FDG can therefore be used to test the efficacy of radiation therapy, including preclinical animal studies. Serial studies are potentially useful to determine if the therapy inhibits tumor metabolism and growth.

A tumor starts to grow from a single transformed cell. This is also the case for tumors used in our preclinical research that

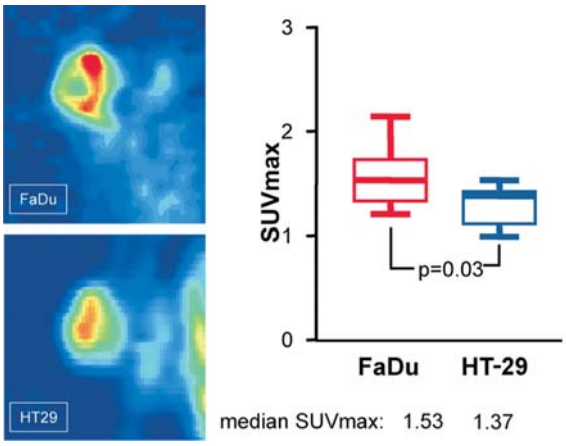

Fig. 1: Schema of the [^{18}F]FDG-uptake in the cells. The glucose transporters transport the neutral [^{18}F]FDG through the cell membrane, like glucose. The hexokinase phosphorylate the [^{18}F]FDG using adenosine triphosphate (ATP) to adenosine diphosphate and [^{18}F]FDG-6-phosphate, which is an anion that remains inside the cells. The accumulation of [^{18}F]FDG in the cells is mainly correlated to the functional expression of the glucose transporters and the hexokinase.

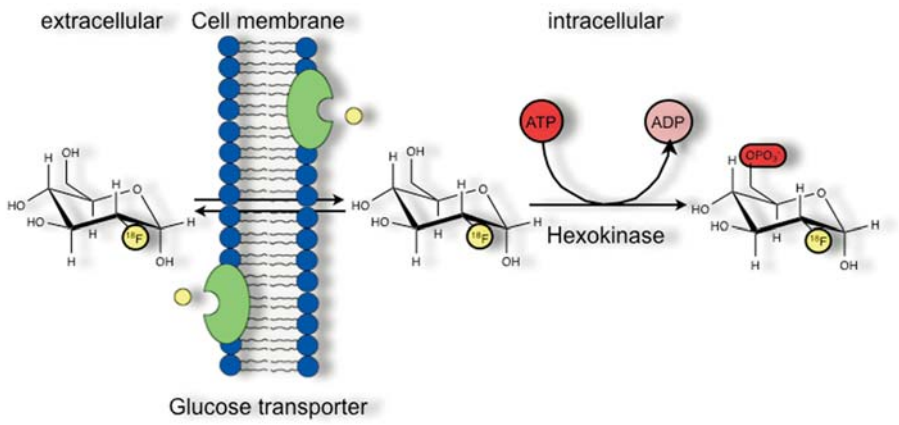

Fig. 2: [^{18}F]FDG distribution in HT-29 and FaDu tumors one hour after injection.

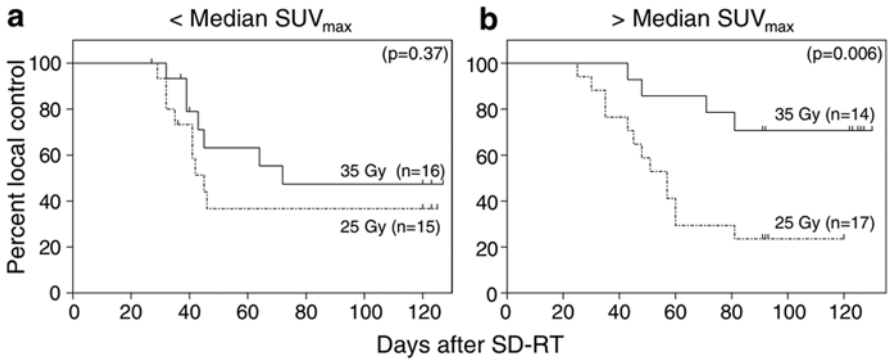

Fig. 3: Actuarial local control rates for FaDu-tumors after irradiation with single doses under ambient conditions stratified according to FDG uptake. Tumors with SUVmax **(a)** below median value, **(b)** above median. Solid lines: 35 Gy, dotted lines: 25 Gy.

were transplanted to immunocompromised mice. However, shortly after the tumors had been transplanted to the host animals, they differed considerably in size, in the localization of the growing tissue inside the tumors, in the amount of blood vessels in the tumors, in oxygen consumption, and other functional and molecular parameters. Increased glucose accumulation in the tumor is associated with the rate of transport across the cell membrane, the activity of hexokinase, and the rate of dephosphorylation in the tissue. The transport of [^{18}F]FDG across cell membranes is mediated by structurally-related proteins constituting a family of glucose transporters, Glut-1 to Glut-5. Significantly elevated expression levels of Glut-1 and Glut-3 are believed to contribute to the accumulation of [^{18}F]FDG in malignant tumors. It has also been suggested that the activity level of hexokinase-II (HKII) influences [^{18}F]FDG accumulation in various malignant tumors. Tumor tissues also show intratumoral heterogeneity in their various properties which may originate from the diverse phenotypic properties of tumor cells or may be induced by their metabolic microenvironment. In this regard, intratumoral heterogeneity of [^{18}F]FDG distribution has been well demonstrated. However, there has been little information on the biologic mechanisms involved in the intratumoral heterogeneous distribution of [^{18}F]FDG. Moreover, the relationships between the intratumoral distribution of [^{18}F]FDG and the response to X-ray therapy remain to be investigated. These data provide the biological basis for diagnosing, staging, and prognosticating malignant tumors and monitoring therapy response by [^{18}F]FDG.

Our objective was to investigate the radiation response of tumors with identical genetic background in relation to the amount and heterogeneity of [^{18}F]FDG uptake (Fig. 2). Research has been partly supported by the EU BioCare project ("Molecular Imaging for Biologically Optimized Cancer Therapy"). Human head and neck squamous cell carcinoma (hSCC; origin FaDu cells) with a diameter of 7 mm xenotransplanted to nude mice were included in the investigations. The tumor

uptake of [^{18}F]FDG was measured without anesthesia immediately prior to irradiation as well as one, four, and eleven days after irradiation. The radiotracer uptake was determined as a maximum standardized uptake value (SUVmax), i.e., the concentration of [^{18}F]FDG in the tumor normalized to the injected dose and to the body weight. This allows a comparison of the data of animals with different body weights. Single-dose irradiations with low and high amounts were applied under normal blood-flow conditions using X-rays. The mice were observed for 120 days after irradiation. This long time period guarantees identification of the reaction of the tumor to therapy, for instance whether the tumors have shrunk, which is quantified as tumor control. This is an authentic "clinical" end point and not a surrogate. In analyzing the animals, the tumor control probability after irradiation with the lower dose (25Gy) was significantly lower than after irradiation with the higher dose (35Gy). The animals were divided into two groups, i.e. animals with pretreatment [^{18}F]FDG uptake in the tumor higher than the median SUV_{max} (1.59) and the group with [^{18}F]FDG uptake in the tumor lower than this. In tumors with [^{18}F]FDG uptake less than the median SUV(max), local control was 37 % after 25Gy whereas it amounted to 47 % after 35Gy. In contrast, substantial differences in local tumor control were found in tumors with FDG uptake above the median SUV(max) (Fig. 3). Multivariate Cox analysis revealed a significant decrease of the recurrence hazard with an increasing dose and SUV(max).

To characterize the functional differences and relations in the tumor tissue, the [^{18}F]FDG uptake was also compared on the tissue level by coregistration of quantitative autoradiography and functional histological images. The [^{18}F]FDG uptake in xenotransplanted squamous cell carcinoma was higher than in human adenocarcinoma (HT-29 cells) (Fig. 2). In hSCC tumors, the [^{18}F]FDG uptake was increased in hypoxic and proliferating tumor regions compared to vital regions without signs of hypoxia and proliferation. However, the [^{18}F]FDG uptake in human adenocarcinoma did not show any dependence on the microenvironment in the vital tumor regions.

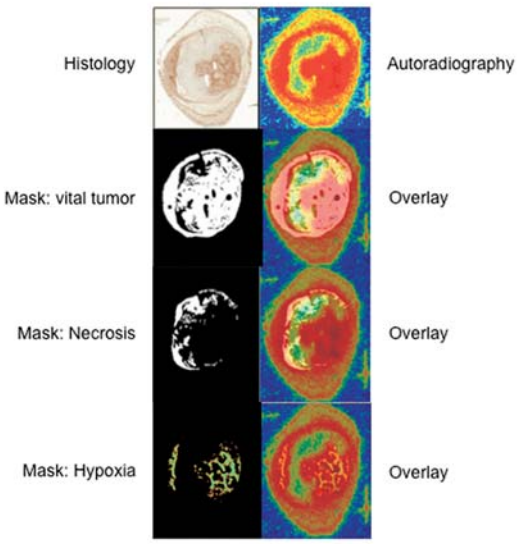

Fig. 4: Autoradiographic and functional histological images of hSCC tumor sections. The images represent the [^{18}F]FDG-uptake (autoradiography), the histology, and the marked regions as masks for the vital, necrotic, and hypoxic regions (left). The right side shows the overlaid masks with the autoradiographic image.

The characterization of the tumor heterogeneity by animal PET in xenotransplanted mice has been extended to the imaging of hypoxia using [^{18}F]Fluoromisonidazole ([^{18}F]FMISO). These investigations are still underway. In summary, tumors are very heterogeneous, not only with regard to their various origins, but also in terms of intratumoral differentiation. We were able to show that hSCC and adenocarcinoma differ in the [^{18}F]FDG uptake and the development of hypoxic regions (Fig. 4). Due to this, tumors also responded differently to radiation therapy. Moreover, we could show that in our model systems, the [^{18}F]FDG uptake is related to the therapy response of the tumors. This supports the hypothesis that pre-treatment [^{18}F]FDG-PET may provide useful information for individual treatment; specific radiation doses can be prescribed to single tumor areas.

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Project partners

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[2]Department of Nuclear Medicine, Faculty of Medicine, Technische Universität Dresden, Germany

Neuroendocrine hormone receptors
as molecular targets for cancer diagnosis and therapy

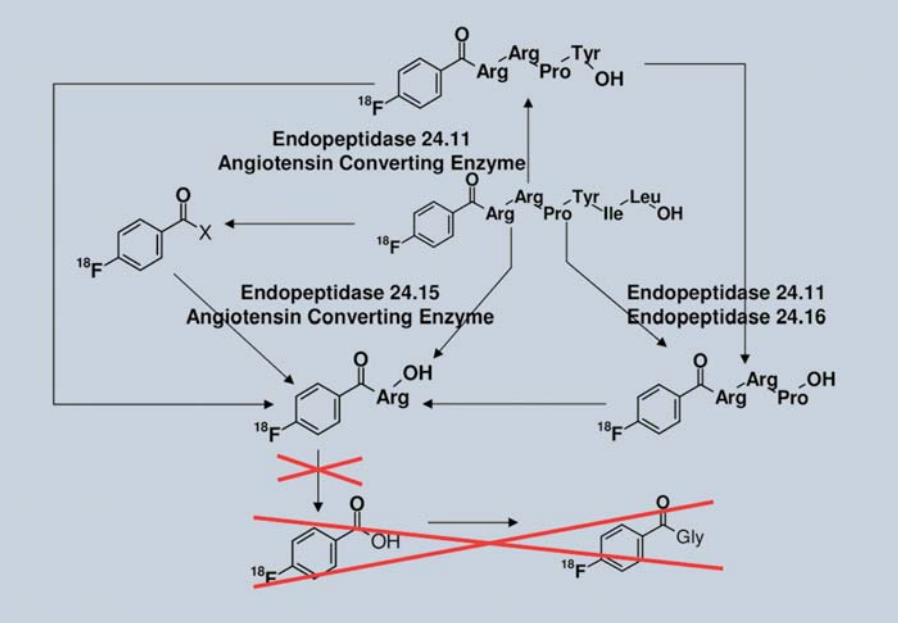

Fig. 1: Metabolism of [^{18}F]FB-NT(8-13).

Ralf Bergmann, Frank Wuest, Jens Pietzsch

Neurotensin and its receptor subtypes

Experimental and clinical data indicate that G-protein-coupled peptide hormone receptors such as neurotensin (NT) play a crucial, but often not fully-appreciated role in the genesis of tumors and metastases. The imaging of these G-protein-coupled peptide hormone receptors and the functional information derived from it will assist in tumor localization, define or predict tumor histology, identify metastases, and plan, guide, and monitor therapy [1]. A particularly devastating disease with very poor prognosis is the pancreatic ductal carcinoma. This tumor urgently requires novel approaches for precise and more effective molecularly-targeted diagnosis and therapy. As this tumor expresses NT receptors, we launched a research project aimed at developing new radiopharmaceuticals for molecular imaging of these neurotensin receptors [2 – 7]. This long-standing project was initiated within the EU BIOMED 2 framework and has created a scientific network of fruitful collaboration with various European partners, particularly with the Department of Organic Chemistry of the Vrije Universiteit Brussels.

Neurotensin is a tridecapeptide (pGlu^{1}-Leu^{2}-Tyr^{3}-Gly^{4}-Asn^{5}-Lys^{6}-Pro^{7}-Arg^{8}-Arg^{9}-Pro^{10}-Tyr^{11}-Ile^{12}-Leu^{13}-OH) produced in the central nervous system. It is mainly found in the gastrointestinal tract in peripheral tissues. The pharmacological effects of neurotensin [2] result from the specific interaction of the peptide with cell-surface neurotensin receptors (NTR). Neurotensin receptors are described as two G-protein-coupled receptors NTR1 and NTR2, and the non-G-protein-coupled receptor NTR3 which is identical with gp95 sortilin. Neurotensin is rapidly degraded in blood plasma by endogenous peptidases and proteases. Several proteolytic enzymes have been reported to cleave intact neurotensin.

Initial studies were aimed at the metabolic fate of neurotensin. For this purpose, the N-terminus of the NTR-binding NT(8-13) fragment was successfully radiolabeled with *N*-succimidyl-4-[^{18}F]fluorobenzoate ([^{18}F]SFB). Several metabolites could be identified, whereas expected catabolites or decomposition products like [^{18}F]fluorobenzoic acid and [^{18}F]fluoride were not detected in plasma or urine. As a consequence, we proposed a new metabolism scheme of [^{18}F]FB-NT(8-13) based on these data (Fig. 1) [2].

Neurotensin receptors in cancer

Neurotensin receptors 1 and 3 are expressed in various tumor cell lines including small cell lung cancer, neuroblastoma, pancreatic, and colonic cancer (HT-29) (Fig. 2). Binding and uptake studies on human adenocarcinoma tumor cell lines showed that after interaction with the peptide, substantial amounts of the receptors were internalized (Fig. 3). Visualization of binding and internalization of a fluorescent probe of a neurotensin analogue were carried out by confocal microscopy (Fig. 4). The investigations were complemented additionally by two-dimensional mass spectrometry of tumor sections (Fig. 5). As is generally known, neurotensin receptors occur abnormally often in a variety of primary human tumors, such as meningioma, Ewing's sarcoma, and ductal pancreatic carcinoma. But neurotensin receptors are also found, though less

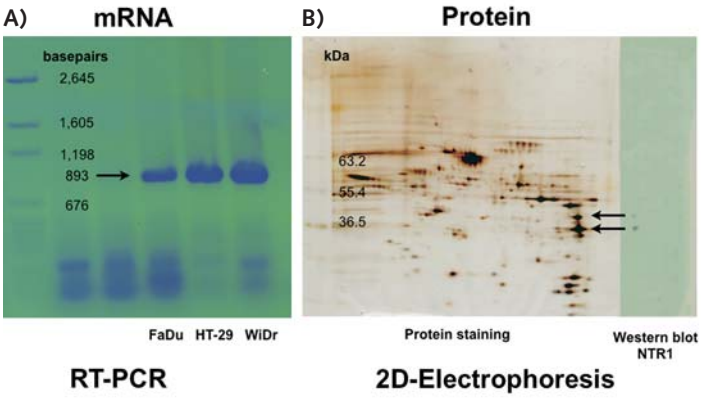

Fig. 2: Expression of NTR1 in cancer cell lines on (**A**) mRNA level in FaDu, HT-29, WiDr and (**B**) protein level using 2D-electrophoresis of HT-29 cell proteins.

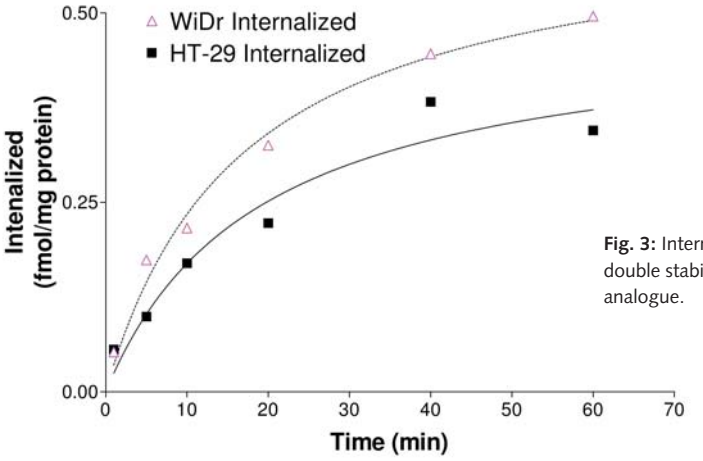

Fig. 3: Internalization kinetics of a double stabilized ^{18}F-labeled NT-analogue.

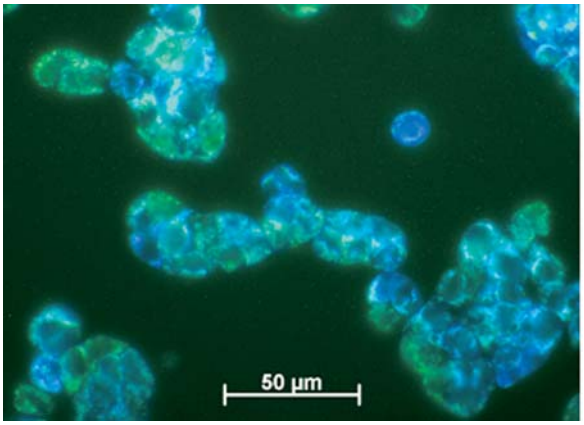

Fig. 4: Localization of a fluorescence-labeled NT analogue (green) on HT-29 cells (autofluorescence, blue) (by courtesy of Thomas Hanke, Institute of Materials Science, Technische Universität Dresden).

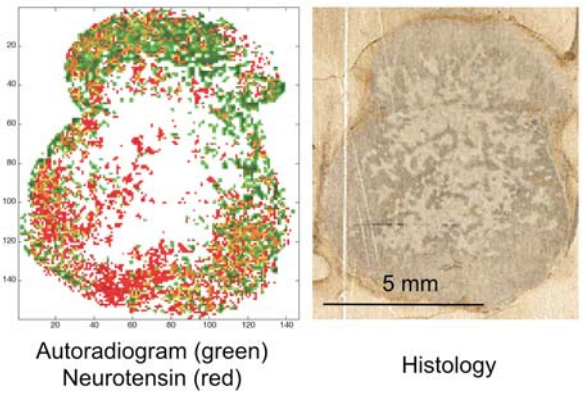

Fig. 5: Image fusion of the autoradiogram of ^{3}H-labeled NT (green) and the distribution of NT(8-13) measured by 2D mass spectrometry (MALDI-TOF; red) on a HT-29 tumor section (left) and the corresponding histological image (right), cooperation with Gerald Steiner and Rainer Salzer (Institute of Analytical Chemistry, Technische Universität Dresden).

often, in astrocytoma, medulloblastoma, medullary thyroid cancers, and small-cell lung cancers. These neoplasms display NTR1 receptor proteins, as well as NTR1 mRNA. However, NTR1 has rarely been found in a number of other cancer entities [1]. The neurotensin peptide itself appears to be expressed by numerous tumors or tumor cell lines. For additional biochemical studies, we cloned the NTR1 gene, expressed it in an *E. coli* system, and isolated the protein. The NTR1 protein will be used as target for the production of specific antibodies. Later, we plan to perform targeting studies.

New radiolabeled neurotensin analogues

For the quantitative characterization of NT analogues, we established chemical, radiochemical, biochemical, as well as radiopharmacological and imaging methods. The animal facility of the Institute of Radiopharmacy at FZD is fully equipped for maintaining immunodeficient and genetically-engineered small animals. The necessary tumor models (Fig. 6) were established and produced in close cooperation with the Centre for Radiation Research in Oncology "OncoRay" and the Department of Radiooncology, both located at the Faculty of Medicine of Technische Universität Dresden.

To increase the biological half-life of neurotensin analogues as one prerequisite of higher tumor accumulation, we followed two strategies: (1) synthesis of stabilized peptides by insertion of non-natural amino acids and formation of pseudo-peptide bonds, and (2) development of multivalent NT(8-13) derivatives [4-6]. Moreover, the increased molecular weight will reduce the glomerular filtration in the kidneys. Two of the new multimeric NT(8-13) tetramers [5] showed an *in vitro* binding affinity towards the NTR1 comparable to that of the parent compound NT(8-13) (Fig. 7). In the current project, new labeling approaches based on prosthetic group chemistry and metal complexes for positron and single-photon emission tomography will be used to perform extensive radiopharmacological studies.

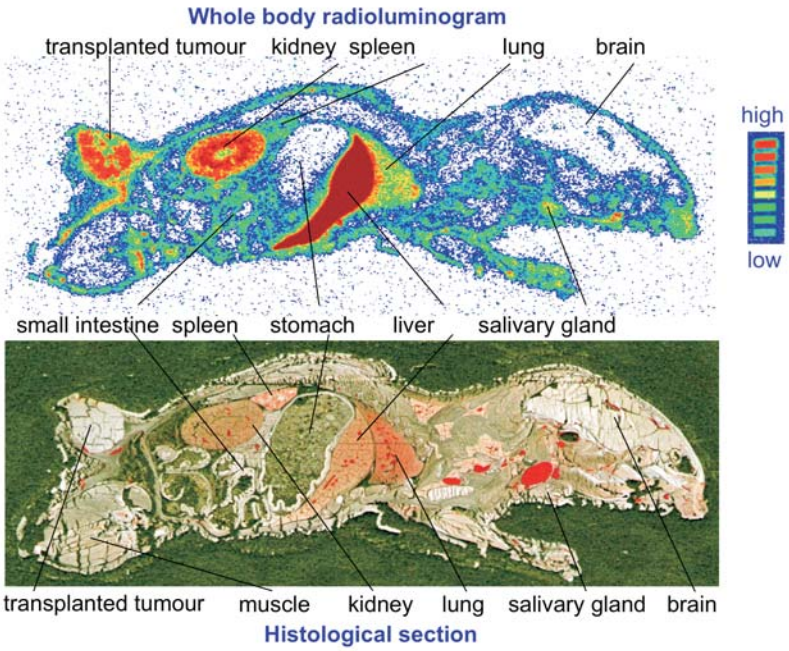

Fig. 6: Whole body autoradiogram of [^{18}F]FB-NT(8-13) analogue distribution and histological section of a HT-29 tumor-bearing mouse.

Future research on neurotensin receptors for diagnosis and therapy

The aim of our future research is to test the following working hypotheses:
(i) functional expression of the NT receptor is characteristic of tumor stem cells,
(ii) newly developed multimeric neurotensin congeners have an increased affinity to the receptors, and (iii) they also show an increased metabolic stability by preserving at least one intact peptide chain for receptor binding if proteolytic degradation in the organism occurs.

The internalization of the agents will allow targeting of intracellular structures with ß-emitting radionuclides. The metal complex analogues are potential agents for therapeutic applications when labeled with ^{90}Y, ^{117}Lu, and ^{188}Re.

The progress made so far with *in vitro* experiments in the model of pancreas tumor (HT-29) and the well-established scientific network are the basis for successful preclinical *in vivo* studies on the role and functional expression of neurotensin receptors in tumors by molecular imaging. This will also lead to additional therapeutic opportunities, especially for small and heterogeneous tumors. Research on the role of the neurotensin receptors in cancer and the development of radiolabeled diagnostics and therapeutics represent an exciting challenge and should thus be extended to other neuroendocrine human receptors such as the neuropeptide y receptor system.

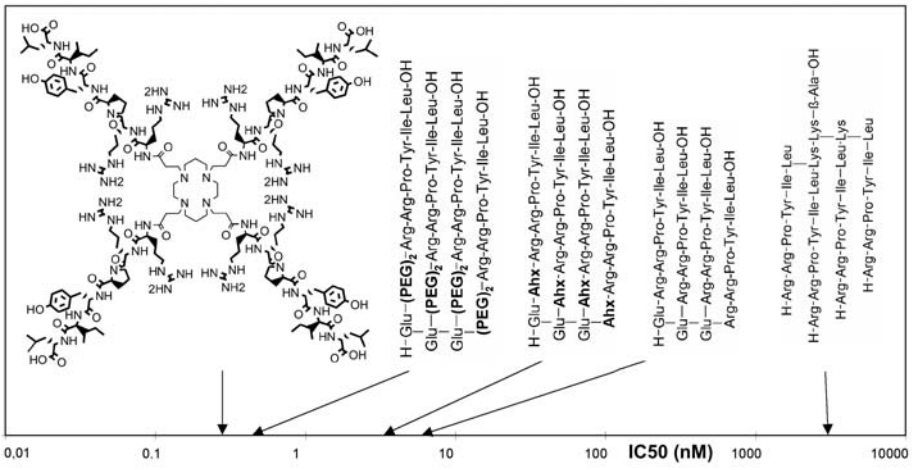

Fig. 7: IC50 of tetrameric NT(8-13) molecules [5]. Synthesis and characterization of the complex compound (left; unpublished result) is part of Anne Kretzschmanns's diploma thesis at the Technische Universität Dresden.

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[3]Institute of Pathology, University of Berne, Switzerland
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Protein oxidation and disease

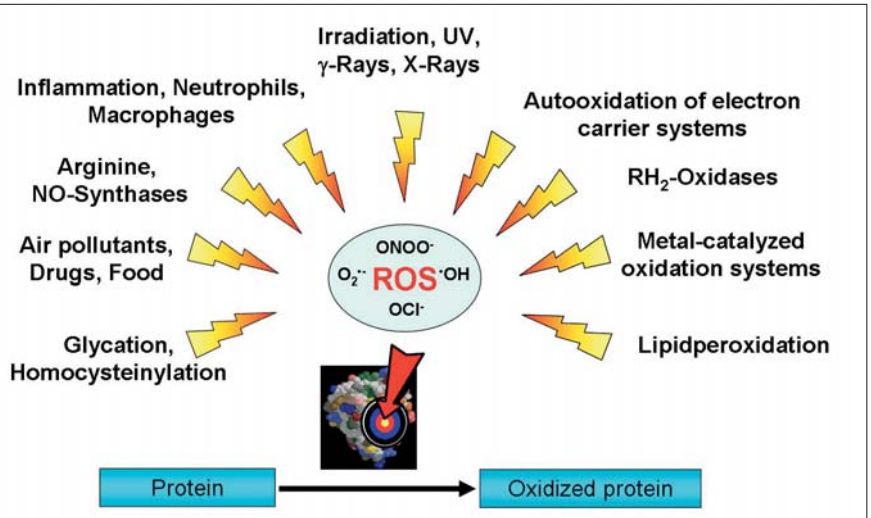

Fig. 1: Generation of reactive oxygen species (ROS) leading to protein oxidation in the human organism. ROS is a collective term including oxygen, nitrogen, or halogen radicals, (e.g., $OH^{\bullet}$, $O_2^{\bullet-}$, $NO^{\bullet}$, $Cl^{\bullet}$) and certain nonradicals (e.g., $^1O_2$, H_2O_2, HOCl, HOBr, $ONOO^-$, Maillard reaction products, homocysteine thiolactone) that are oxidizing, nitrating, halogenating, glycating, or homocysteinylating agents or are easily converted into radicals. ROS and other prooxidative reactive species i) are present as pollutants in the normal atmosphere, ii) are generated as by-products of normal metabolic processes, iii) are formed by bioactivation of xenobiotics, and iv) are formed during exposure to X, γ, or UV radiation.

Jens Pietzsch, Frank Wuest

Controlling the damaging effects of reactive oxygen species (ROS) on proteins is a major unsolved problem in current biology and medicine [1]. In the 1960s, Garrison and others carried out pioneering studies on oxidative modification of proteins exposed to ionizing radiation. Recent progress in life sciences has led to the development of excellent methods and approaches enabling an increasingly detailed investigation of the phenomenon of *protein oxidation* at both a micro and macrostructural level under various physiological and pathophysiological conditions. Recent experimental and clinical evidence underpins the argument that protein oxidation is a significant causative or associated factor in aging as well as in the etiology, progression, and manifestation of a panoply of human diseases and disorders (Table 1) [1]. Protein oxidation is defined here as covalent modification of proteins induced either directly by reactive oxygen species or indirectly by reactions with secondary by-products of oxidative stress (Fig. 1). Proteins are the major target of reactive oxygen species due to their abundance in biological systems as well as their high rate constants for reaction [1]. Protein oxidation may be important *in vivo* for two reasons: Firstly, it affects the function of receptors, enzymes, transport proteins, and cell signaling mechanisms. Secondly, protein damage can lead to secondary damage to other biomolecules, e.g., by inactivating DNA repair enzymes.

Most protein damage is non-repairable and has deleterious consequences on the structure and function of proteins. But some oxidation processes can be reversed in some circumstances. This is particularly important because the generation of reactive oxygen species cannot solely be considered as a purely pathophysiological phenomenon. When nitric oxide ($NO^{\bullet}$) was identified as an endothelium-derived relaxing factor, it became incontrovertible that reactive oxygen species are essential entities in mammalian biochemistry. Reversible protein oxidation is thus a routine, purposeful aspect of the cell's normal function [1]. In order to understand how protein oxidation causes disease, it is important to find out which proteins are affected by reactive oxygen species and to what degree they are modified *in vivo*. Of further interest are the specific functional consequences of protein modification and the critical 'hits' of reactive oxygen species that are relevant in the etiopathology of diseases.

Table 1: Human disorders and diseases associated with increased protein oxidation:

· Acute respiratory distress syndrome	· Cancer	· Infectious diseases	· Osteoarthritis
· Alcoholic liver disease*	· Diabetes mellitus (and complications)*	· Inflammatory bowel diseases*	· Parkinson's disease
· Alzheimer's disease	· Glomerulonephritis*	· Injury	· Progeria
· Amyotrophic lateral sclerosis	· Hypertension*	· Nephrotic syndrome*	· Rheumatoid arthritis*
· Atherosclerosis*	· Impaired glucose tolerance (Prediabetes)*	· Obesity*	· Sepsis

The asterisk denotes diseases that show a relevant involvement of modified lipoproteins in their pathogenesis.

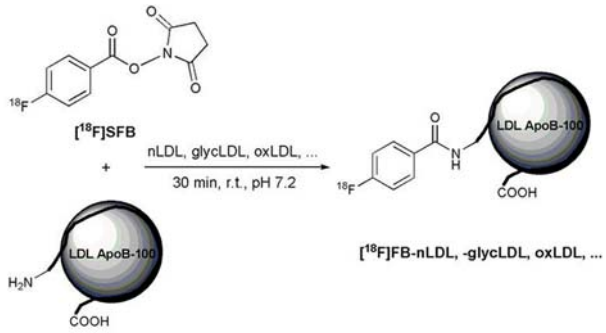

Fig. 2: Reaction scheme for fluorine-18 radiolabeling of native and modified low density lipoproteins (LDL) with the amino group reactive agent N-succinimidyl-4-[^{18}F]fluorobenzoate ([^{18}F]SFB) under mild reaction conditions. ApoB-100 denotes the apolipoprotein B-100, the major structural protein of human LDL.

These issues are in the heart of research at the FZD Institute of Radiopharmacy, which collaborates closely with the Department of Internal Medicine at the Faculty of Medicine Carl Gustav Carus of Technische Universität Dresden. The aim is to provide novel targets for further research into the disease process as well as sites of potential therapeutic interest. In particular, we have intensively studied the oxidative modification of apolipoprotein B-100, the major protein of human low-density lipoproteins (LDL). Due to the *"oxidative modification hypothesis of atherosclerosis"* [1], oxidative modification of apolipoprotein B-100 by reactive oxygen species is widely regarded as a crucial event in the atherogenic process. Only recently could we provide new evidence supporting the role of oxidative damage of apolipoprotein B-100 in the pathogenesis of impaired glucose tolerance, a prediabetic state marking an important facet of the Metabolic Syndrome [2]. The major finding of this study is that low density lipoproteins obtained from subjects with impaired glucose tolerance exhibited an increased apolipoprotein B-100 glycoxidative status when compared with low-density lipoproteins from normoglycemic subjects. Low-density lipoprotein modifications were closely correlated with sustained baseline hyperglycemia observed in subjects with impaired glucose tolerance as well as with temporary hyperglycemia after an oral glucose challenge. As a consequence of apolipoprotein B-100 modification, low-density lipoproteins from subjects with impaired glucose tolerance evoked a significantly altered expression of certain scavenger receptors and peroxisome-proliferator activated receptors, respectively, in macrophages. In conclusion, impaired glucose tolerance is causally related to glycoxidative modification of circulating low-density lipoproteins. Also, it is associated with early events in the pathogenesis of cardiovascular complications in prediabetic and diabetic patients. These data further underline the importance of early preventive treatment and strategies [2].

Despite the evidence from clinical studies, however, the role of circulating modified low-density lipoprotein particles in the *in vivo* development of atherosclerosis is still a matter of debate. It could be argued that perhaps an increased level of circulating oxidized low-density lipoproteins is an epiphenomenon that merely shows correlation with the basic pathological or degenerative processes or with impairment of antioxidant barriers. On the other hand, circulating oxidized low-density lipoproteins, e.g., descending from an inflammation site as the inflamed joints of subjects suffering from rheumatoid arthritis could be a causative substrate for the development of a pathological situation in another compartment, such as lesion formation in the arterial wall [1]. Therefore, we developed highly sensitive

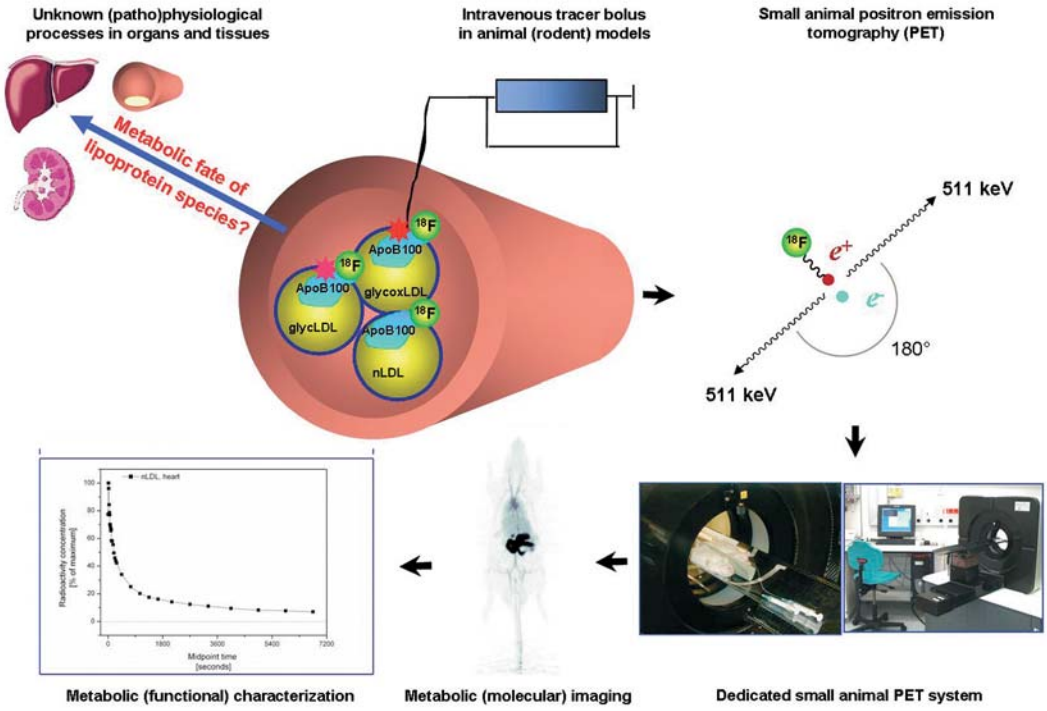

Fig. 3: Study design and principle of small animal positron emission tomography studies aiming at characterization and differentiation of the metabolic fate of native and modified low density lipoproteins in animal (rodent) models of disease and controls.

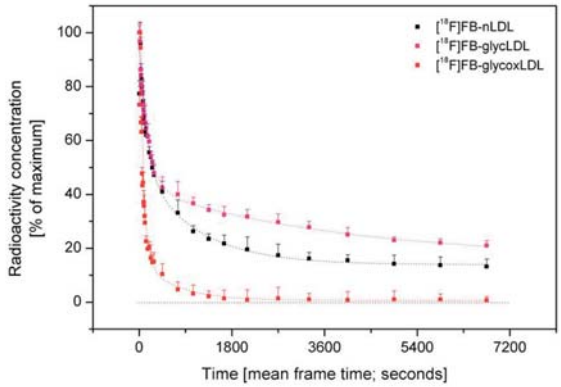

Fig. 4: Representative time-activity-curves showing kinetics of the fluorine-18 radioactivity concentration during 120 min after injection of radiolabeled ([^{18}F]fluorobenzoylated, [^{18}F]FB-) native low density lipoproteins (LDL), glycated LDL, and glycoxidized LDL as calculated from region-of-interest analysis of dynamic small animal positron emission tomography scans of the heart (mainly representing the cardiac blood pool). Results are expressed as means ± SD of five independent experiments. Dynamic positron emission tomography data demonstrated a significantly delayed catabolism of glycated LDL compared with native LDL. In contrast, glycoxidized LDL showed an enhanced catabolism when compared with native LDL [1].

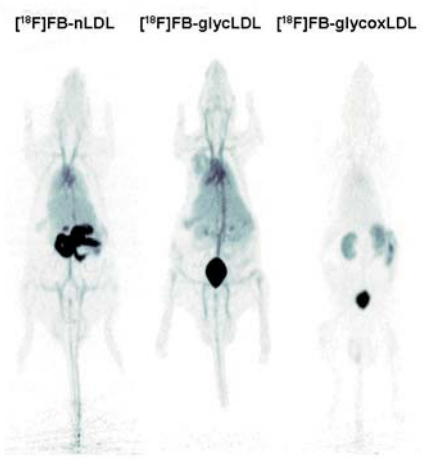

Fig. 5: Representative whole body small animal positron emission tomography scans (maximum intensity projection) showing the metabolic fate of fluorine-18 radiolabeled ([^{18}F]fluorobenzoylated, [^{18}F]FB-) native and certain modified low density lipoprotein species (high radioactivity concentration is indicated by black color) in a rat model at 180 min post injection.

and specific methods for radiolabeling of native and modified proteins with the positron emitter fluorine-18. This allows initial direct assessment of intravascular transfer and catabolism of modified low-density lipoproteins using dynamic small animal positron emission tomography in rodent models *in vivo* (Fig. 2 – 3) [1, 3, 4]. In this regard, our experiments showed an extremely fast and complete blood clearance of glycoxidized low-density lipoproteins *in vivo*. This has been explained by the concerted action of various scavenger receptors on resident macrophages and endothelial cells in liver, spleen, and kidney (Fig. 4). These cells form an efficient scavenger apparatus protecting the organism from the atherogenic action of glycoxidized/ oxidized low-density lipoproteins in the circulating blood compartment.

Other circulating modified low-density lipoprotein species, e.g., glycated LDL, avoid this scavenger pathway and are rerouted to various tissue-specific proinflammatory pathways [1 – 4]. The *in vivo* distribution and kinetics of both native low density lipoproteins and modified low density lipoproteins correlated well with the anatomical localization and functional expression of low density lipoprotein receptors, scavenger receptors, and receptors for advanced glycation end products. Interestingly, the vasculature in rodents shows a substantial temporary retention of glycated low-density lipoproteins when compared with native or glycoxidized low-density lipoproteins. This retention cannot be explained by the circulating blood pool or perfusion effects alone. Therefore, it is indicative of a tissue-specific interaction with glycated low-density lipoproteins that is potentially proatherogenic (Fig. 4 – 5) [5]. Given the intrinsic properties of small animal positron emission tomography, our approach enables first-time quantitative characterization and discrimination of the kinetics and the metabolic fate of native and oxidatively modified proteins, and, *vice versa*, of functional expression of their pathophysiologically relevant tissue-specific binding sites *in vivo* [5]. Ongoing studies in animal models of disease funded by the German Research Foundation (DGF) will thus provide detailed information on the importance of circulating modified lipoproteins in the etiology, progression, and manifestation of prediabetes, the Metabolic Syndrome, and other pathologies.

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Radioactive metals for tumor therapy

Preparation of radiopharmaceuticals in hot cells. Photo: Jürgen Lösel

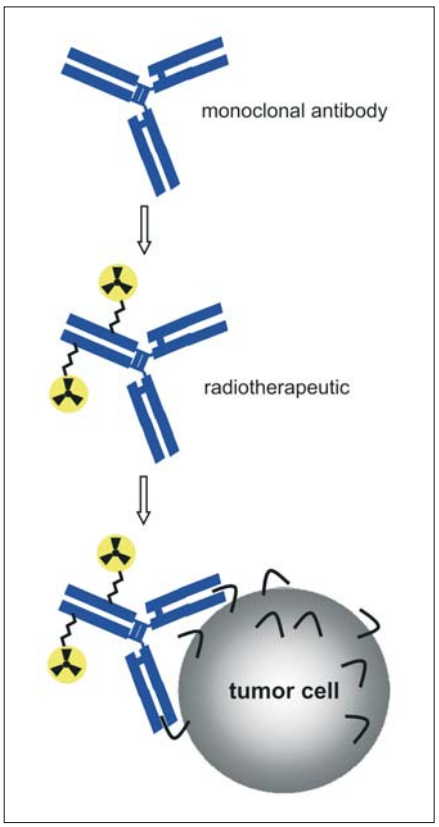

Fig. 1: Principle of radioimmunotherapy: a radiotherapeutical clings to a tumor cell where radioactive radiation unfolds its cell-destructive effect.

Hans-Jürgen Pietzsch, Holger Stephan, Jörg Steinbach

Ionizing rays, after the surgeon's knife, are the most successful and most frequently-applied weapon against cancer. In external radiotherapy, high radiation doses are concentrated onto a small area in the body. This aims at destroying the pathologically modified body cells in the tumor whereas, at the same time, the adjacent healthy tissue is spared. However, external radiation is limited by metastasing conditions. In these cases, the treatment method must be systemic, i.e., therapeutic tumor agents must reach the (partly invisible) metastases and solid tumors via the bloodstream. This is the field of chemotherapy and targeted internal radionuclide therapy, which make use of particle radiation emitted by radionuclides (usually beta radiation) as a therapeutically effective dose. In the course of treatment, the radiolabeled substance is transported to the tumor and the radiation energy released there causes the tumor cells to die off. The main challenges of this therapy are targeting the tumor and the time distribution of the radioactivity. Radioimmunotherapy (RIT) as a special form of endoradionuclide therapy has gained in clinical importance. With it, antibodies specifically targeting tumor cells are labeled radioactively. Together with the antibodies, the radionuclides arrive at the tumor where their radioactive radiation can deploy its cytocidal effect. In doing so, healthy tissue is largely spared (Fig. 1). Radiotherapeutical applications require radionuclide-emitting particles (beta and alpha particles), the half-life periods of which range from several hours up to a few days. Still, a high local radiation dose is generated. As a consequence, there should be sufficient time to prepare the medication, transport it to the tumor and, above all, release the radiation dose into the tumor cells. However, in order to

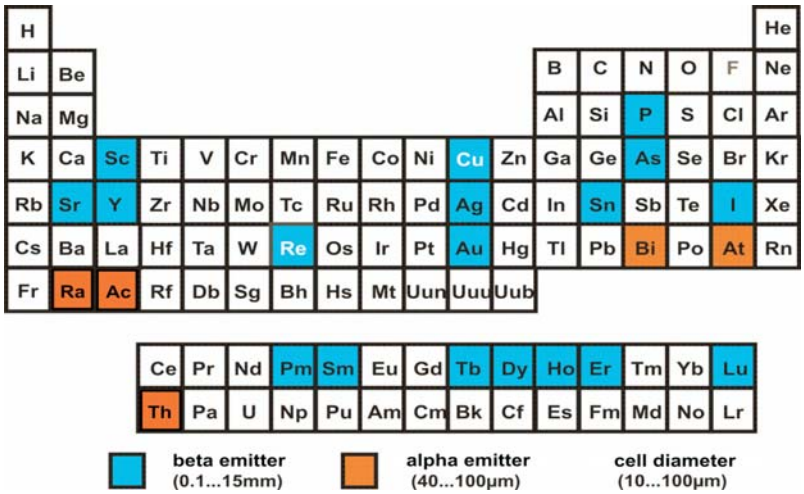

Fig. 2: Survey of elements the radioisotopes of which are potent therapeutical nuclides.

minimize the exposure of healthy tissue to radiation, the radionuclides must decay within a relatively short time into non-radioactive, i.e., stable daughter nuclides. Fig. 2 presents a survey of elements the radioisotopes of which are potent therapy nuclides.

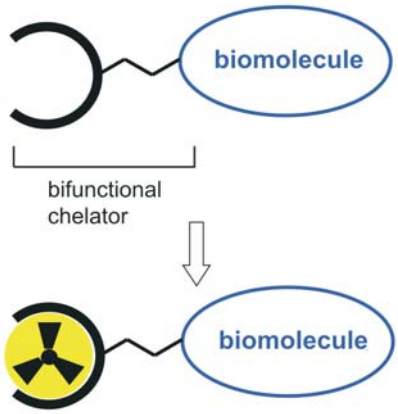

Fig. 3: Principle structure of a radiometal pharmaceutical
Basically, a radiometal pharmaceutical consists of four components: a bifunctional chelate group, a spacer unit, a radionuclide, and a biomolecule. The bifunctional chelate group must bind the radionuclide in a most stable manner and contain a couplable group to tie biomolecules. Biomolecules, such as specific peptides, proteines, antibodies, or aptameres, are linked via a spacer to the bifunctional chelate unit and are expected to enable controllable biodistribution. Labeling the chelate unit equipped with the biomolecule by means of the radionuclide shall preferably be carried out at the last stage in order to facilitate the user to apply a simple single-step approach.

We started our research using the radionuclides copper-64 (Cu-64), copper-67 (Cu-67), and rhenium-188 (Re-188) with half-life periods of approximately 13, 62, and 17 hours, respectively. Apart from the desired particle radiation, these radionuclides simultaneously emit positron or gamma radiation, which by means of special detection techniques allows registering the distribution of radioactive substances and visualizing them. Fortunately, these radionuclides are easily available. For instance, we can produce Cu-64 with the help of our own cyclotron. For obtaining Re-188, we use a commercial radionuclide generator which can also be installed in hospitals. As the maximum range of radiation in tissue varies between about 1 millimeter for both Cu-64 and Cu-67, and 11 millimeters for Re-188, tumors of various sizes can be targeted.

Therapeutical application of radionuclides requires radiopharmaceuticals to have a high metabolic and radiolytic stability. This means that the compound applied must stay intact until it has reached the desired place where it should remain until its radiation has abated. Possible metabolites of these radiopharmaceuticals must leave the body without damaging healthy tissue. Thus, we are searching for compounds which incorporate a complex-forming section for metallic radionuclides to fulfill several tasks (Fig. 3). Due to the differing coordination chemistry of rhenium and copper compounds, both radiometals require a differentiated approach. Currently, we are pursuing two approaches to develop radiolabeled rhenium and copper compounds:

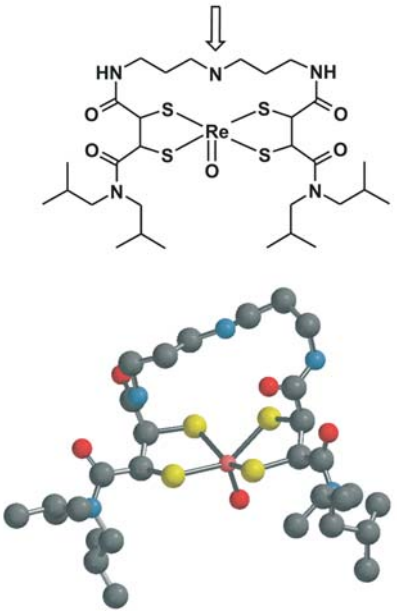

Fig. 4: Rhenium complex on the basis of bridged dimercaptosuccinic acid chelate units.

Radiolytically resistant Re-188-S_4 chelates for labelling biomolecules

Chemically very robust radioactive rhenium complexes can be generated on the basis of bridged dimercaptosuccinic acid chelate units (Fig. 4), permitting wide structural variety as well. Furthermore, solubility-mediating units can be tied to the carboxyl groups of the dimercaptosuccinic acid. Yet synthesizing such rhenium complexes is a challenge to chemists as stereoisomers are generated during production, i.e., compounds with the same constitution, but with a different spatial, three-dimensional arrangement of their atoms and atom groups [1, 2].

The Re-188 labeling procedure runs quickly in good yields and under mild conditions, such as aqueous solution, neutral pH, and room temperature. The appropriate Re-188-S_4 complexes were found to be very stable regarding re-oxidation and ligand exchange *in vitro*

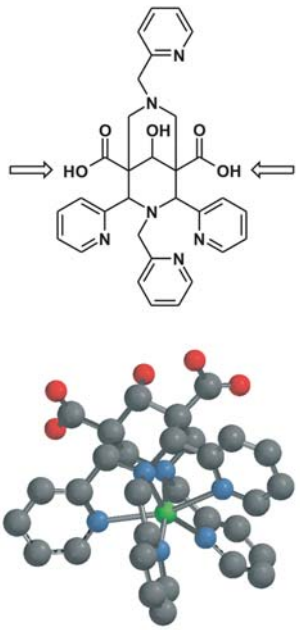

Fig. 5: Bispidine ligand of four pyridine groups and the appropriate copper(II)-complex.

and *in vivo*. All in all, the new Re-S_4 complexes offer the possibility of stable and highly-specific activity labeling of biomolecules for therapeutic applications [3, 4].

Highly stable complexes of radioactive copper nuclides with bispidines

We are developing a ligand system with colleagues from the Institute of Inorganic Chemistry at Heidelberg University which is suitable for generating extremely stable copper(II)-complexes. Fig. 5 shows one representative. Here, the Cu(II) ion is bound by a total of six donor atoms (two amine nitrogen and four pyridine nitrogen atoms) and is practically completely shielded from its environment, which explains its high stability. Studies during which these ligands were labeled with copper radionuclides indicate a rapid formation of stable complexes under mild conditions. Furthermore, the bispidine structure opens suitable chemical approaches to introduce biomolecules, which are important in view of the targeting of such complexes. Due to these promising features, bispidines are predestined as attractive candidates for developing new copper-based radiopharmaceuticals [5].

Principally, the concepts presented here may also be applied to the design of metal complexes of other therapeutically relevant radionuclides. For this purpose, a multitude of subtasks, particularly with regard to research on ligand synthesis, coordination chemistry, as well as tumor-biological and radiopharmacological aspects, must be solved.

Radionuclide therapy is challenging, but still evolving in regards to the investigation of new molecular constructs, new radionuclides and radiochemistry, improved dosimetry, prediction of tumor response and host toxicities, and better targeting strategies to prevent or overcome host toxicities, particularly liver and kidney toxicity and myelosuppression. Hopefully, the advances in radioimmunotherapy regarding hematologic malignancies will translate to progress in the therapy of radioresistant solid tumors.

In the field of developing radiometal compounds for tumor therapy, we are enjoying external collaboration with experts at the Paul Scherrer Institut (Switzerland), and at the universities in Dresden, Heidelberg, and Padua (Italy). In the future, these efforts will be intensified strongly in the context of "OncoRay"—Centre for Radiation Research in Oncology, which is jointly run by Technische Universität Dresden, FZD, and University Hospital Carl Gustav Carus in Dresden.

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Medical impact and beauty of cluster compounds

Holger Stephan, Gerhard Geipel

Polynuclear metal compounds may have considerable potential as metallic drugs. The most prominent representatives are polyoxometalates, which have been under investigation since the last third of the 19th century. They contain transition metal ions such as tungsten, molybdenum, vanadium, and so on which are bridged by oxygen atoms (Fig. 1) [1]. In addition to applications in catalysis, separation, analysis, and as electron-dense imaging agents, some of these substances have been shown to exhibit biological activity *in vitro* as well as *in vivo* ranging from anti-cancerous, antibiotic, and antiviral up to anti-diabetic effects. Yet fundamental questions regarding the mechanism of many of the observed medical effects have remained essentially unanswered. Modern experimental techniques developed in the last decades may help to explore this exciting area. This requires interdisciplinary collaboration between chemists, crystallographers, physicists, biochemists, pharmacists, and physicians.

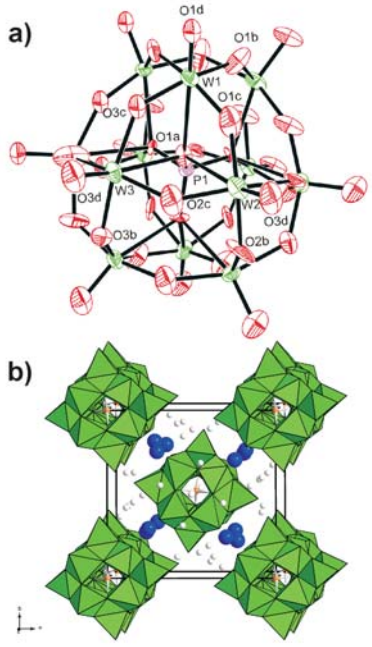

Fig. 1: a) Molecular structure of a typical Keggin type polyanion $[Ti_2W_{10}PO_{40}]^{7-}$ **b)** Crystal packing of $K_4H_3[Ti_2W_{10}PO_{40}] \cdot 15H_2O$, with the $[W/TiO_6]$ octahedral coordination polyeder (green), potassium cations (blue), and water molecules as light gray spheres.

Polymetalates represent a diverse ensemble of nanostructures with almost infinite variability in their chemical, physical, and biological properties. Typical covalent-bridged cluster compounds are sized between 1 and 3 nm, creating fascinating and beautiful molecules (Fig. 1–5). Attaching special surface groups on the periphery of cluster compounds may result in self-assembled non-covalent organized structures larger than 5 nm which are characteristic of bio-molecules such as enzymes. Cells of mammalian organisms are typically between 10 and 30 µm; however, sub-cellular organelle dimensions are smaller—in the sub-µm range. This comparison of size dimensions illustrates that polymetalates are small enough to allow the cell membrane to be penetrated without excessive interference. Evidently, some types of polymetalates can be transported into cells, particularly into mitochondria. Our aim is to develop novel cluster compounds with improved chemical and metabolic stability. Furthermore, increased recognition of target biomolecules such as enzymes is a goal as well. Regarding this, the conjugation of organic groups to the inorganic metal cluster compounds could be used to enhance biological targeting. The development of such inorganic-organic hybrids is particularly challenging.

During exploration of biological activity of polynuclear cluster compounds, we recently recognized polyoxometalates as a new class of potent enzyme inhibitors [2]. E-NTPDases (ecto-nucleoside triphosphate diphosphohydrolases) are surface-located nucleotide-hydrolyzing enzymes involved in the regulation of signaling cascades by activating G protein-coupled P2 receptors. Currently, there are significant efforts underway to find efficient and selective inhibitors for these enzymes in order to modulate receptor activity and to influence the pharmacological effects as a consequence. The most potent compound described to date is $K_6H_2[TiW_{11}CoO_{40}]$ exhibiting K_i values which are significantly lower than those of known standard inhibitors (Fig. 2). In future joint experiments with the Pharmaceutical Institute of the Universität Bonn, the nature of the enzyme inhibition mechanism caused by polyoxometalates will be researched. Most importantly, we wish to determine the method of action for the biological effects including their anti-cancer activity.

A promising new class of cluster compounds are hexanuclear rhenium complexes with bridging sulfur, selenium,

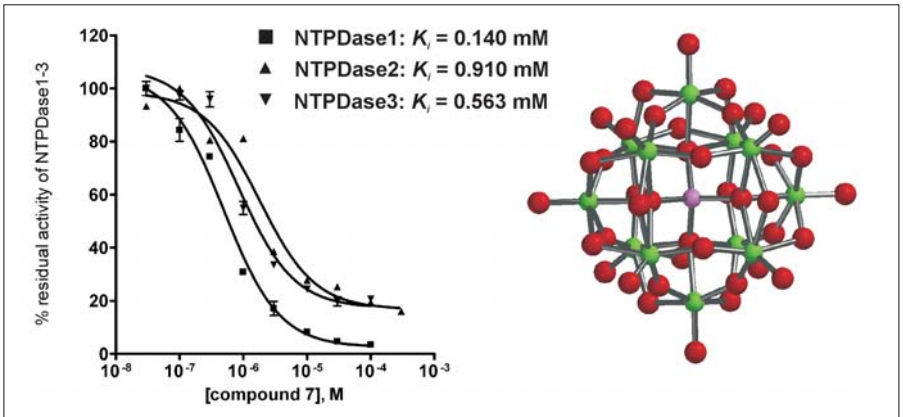

Fig. 2: Concentration-inhibition curves for the enzymes E-NTPDase 1 – 3 with $K_6H_2[TiW_{11}CoO_{40}]$.

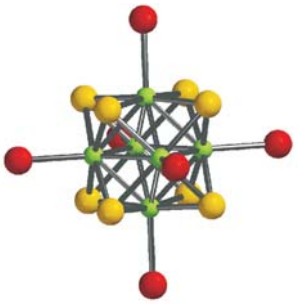

Fig. 3: Molecular structure of the cluster polyanion $[Re_6S_8Br_6]^{3-}$ (rhenium – green, sulfur – yellow, bromine – red).

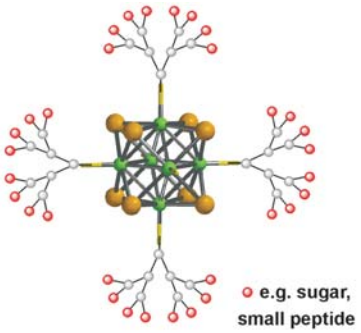

Fig. 5: Cluster compound with pendant dendritic ligands.

and/or tellurium atoms (Fig. 3) [3 – 5]. Increased attention to these complexes is due to their structural, redox, and photoluminescent properties as well as their rich chemistry which derives from a simple modification of their coordination environment. Attaching organic ligands to the cluster environment seems to be very attractive in view of developing biocompatible and bio-available hybrid compounds for therapeutic purposes. For instance, octahedral rhenium complexes with grafted pyrazole ligands possess bright red luminescence (Fig. 4) which makes them attractive for medical treatment of cancer by means of photodynamic therapy. Furthermore, the inherent potential of this novel class of cluster compounds for anti-tumor activity, photosensitizing, and radiation-sensitizing properties can provide synergetic medical efficacy by simultaneously applying various methodologies. Thus, chemotherapy could be combined with photodynamic therapy or radiation therapy.

The enormous versatility and variety of these cluster structures offer considerable opportunity in these areas. In the future, the know-how of our Russian colleagues at the Nikolaev Institute of Inorganic Chemistry in Novosibirsk, Russia in synthesizing appropriate rhenium cluster compounds and our expertise in developing dendritic (tree-like) ligands shall be allied in order to create intelligent vehicles with tunable transport properties. The dendritic modification of polynuclear metal compounds allows the intended structures to have versatile chemical, physical, and biological properties (Fig. 5). Most importantly, both the biodistribution and the biological targeting may be influenced by grafting a multitude of suitable bio-molecules onto the surface of the metal cluster compounds. To achieve this ambitious goal, the Nikolaev Institute of Inorganic Chemistry, the Leibniz Institute of Polymer Research Dresden, and the FZD will collaborate on a project funded by the International Bureau of the Federal Ministry of Education and Research (BMBF). This will focus on the synthesis of novel cluster compounds and the characterization of the luminescence properties by laser spectroscopy. Structural characterization will be performed in collaboration with colleagues from the Federal Institute for Materials Research and Testing (BAM) in Berlin and biological activity will be tested at the Pharmaceutical Institute in Bonn.

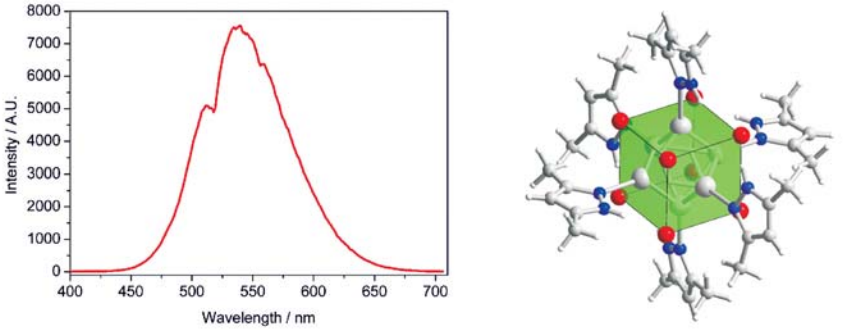

Fig. 4: Luminescence spectra of the cluster compound $[Re_6Se_8L_6]Br_2L_2$ (L = 3,5-dimethylpyrazole).

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Moving targets – correcting patient movement in positron-emission tomography

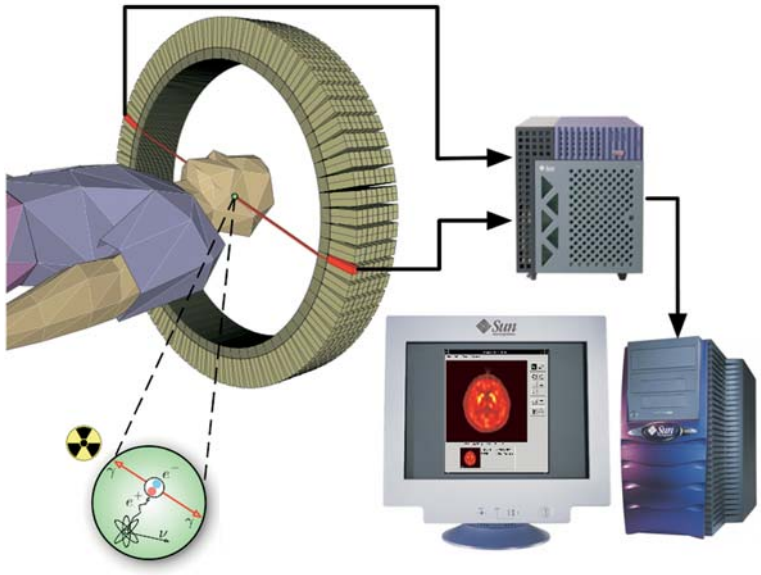

Fig. 1: Schematic overview of measurement process in PET.

Jörg van den Hoff

Positron Emission Tomography (PET) is a tomographic imaging technique which enables quantitative investigation of cellular and molecular processes, such as blood flow, transport across diffusion barriers, substrate metabolism, protein synthesis, enzyme activity, receptor affinity, and so on. Today, PET is firmly established in preclinical and clinical research as well as for clinical applications. The PET technique assesses in vivo the three-dimensional distribution of special substances (tracers) labeled with short-lived positron emitting isotopes. An important example is the sugar 18F-Deoxyglucose (18F-FDG), currently the most widely used tracer for clinical oncological investigations.

The measurement of the regional tracer distribution depends upon detecting the radioactive decay of individual atomic nuclei. Upon the decay of the radioactive label, a positron is emitted from the atomic nucleus and is rapidly stopped in the surrounding tissue (typical mean range in water < 1 mm). The positron then annihilates with an electron, which is accompanied by collinear emission of two 511 keV photons in opposite directions. Thereupon, these photons are detected with several thousand scintillation detectors arranged in a ring coaxial to the patient's body axis. The coincident detection of two photons within a time window of a few nanoseconds defines the so called Line of Response (LOR) along which the primary decay of the radioactive label has occurred. In other words, this coincidence measurement provides the directional information for the subsequent tomographic reconstruction of the tracer distribution (Fig. 1).

The resulting tomographic images quantitatively provide the regional concentration of the applied tracer substance. Yet PET requires long-term data acquisition—from several minutes up to more than one hour. During this "exposure time", patient movements are unavoidable and deteriorate the image quality, reducing the spatial resolution and creating image artifacts ("ghost images"). As the intrinsic spatial resolution of current PET scanners constantly improves, patient movement increasingly limits the image quality which is practically achievable. As far as the PET's contribution to biologically individualized and technically optimized radiotherapy is concerned—a subject which we are working on with the recently established OncoRay research center in Dresden—reliable elimination of motion artifacts appears highly desirable. Therefore, our group is developing methods for accurate correction of two different types of patient movement: first, cyclic organ and body motion correlated to the heart and breathing cycle and secondly, erratic involuntary movement of the patient.

In order to perform accurate motion correction, several problems must be solved:

1. Monitoring of the patient with external motion tracking devices providing high temporal and spatial resolution,
2. Spatial and temporal synchronization of the motion tracking data with the reference frame of the PET scanner (cross calibration),
3. Modifying the PET data acquisition technique,
4. Development of algorithms for performing the actual motion correction within the PET data.

As far as the first item is concerned, we use a system of infrared video cameras for tracking the motion of targets attached to the patient (Fig. 2). Every 50 milliseconds, the system reports the patient's position with sub-millimeter accuracy. The cross calibration to the tomograph is performed at the same level of accuracy by imaging the motion tracking target with the tomograph using an external radioactive source.
Substantially more effort is necessary with regard to the remaining issues of the

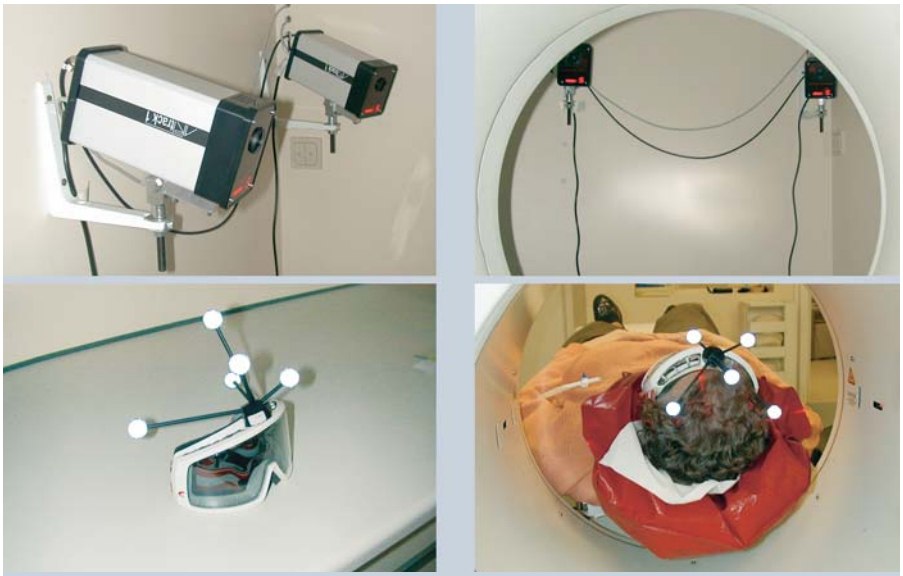

Fig. 2: The motion tracking system for brain applications.

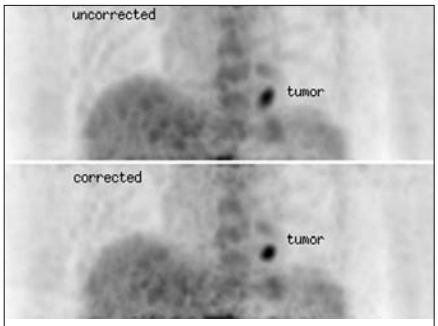

Fig. 3: Correction of breathing cycle-related motion. In the corrected image the tumor is much better delineated and significant errors in assessing the size and metabolic state are avoided.

above list. During routine operation, the coincident events are fed into the data processing electronics of the tomograph and stored in a so-called histogrammic memory. Each Line of Response (LOR) corresponds to a single memory cell containing the cumulative number of events which occurred along this LOR during data acquisition. (This can be compared to the way photographs are stored on the memory cards of digital cameras.) Only after measurement are the histogram data transferred to the computer hard disc. In this way, significant data compression is achieved, but at the price of losing the timing information of the specific events. Since it is exactly this timing information which is needed for motion correction, we use the list mode in which each coincident event is directly stored on the computer's hard disc together with its timing information. (Regarding the example from digital photography, this corresponds to separately storing location and time of the arrival of each individual photon hitting the CCD chip of a digital camera.) Since list mode measurements are not adequately supported by the tomograph's software, we have developed tools from scratch to manage large list mode data sets, notably with regard to integrating multiple trigger signals from breathing monitors and electrocardiograms (ECG). Fig. 3 shows an example of the benefits of using a breathing trigger and list mode data.

The main challenge is to correct the primary list mode data prior to tomographic reconstruction. If this can be accomplished, tomographic images which are completely free of any motion blurring or other motion artifacts could be obtained. For each coincident event, our algorithm computes a reorientation which repositions the LOR to its original orientation within the detector ring. While this reorientation is straightforward, several complications exist. The most obvious are differences in detector efficiencies, discretization and interpolation errors, and, most notably, LORs drifting out of the scanner's field of view and/or reentering the field of view during measurement. Last but not least, efficient implementation of the algorithms on multi-processor systems is necessary so the methods can be used for clinical studies. Fig. 4 shows the reward of using such elaborate methods. This technique is already operational and has been used, for instance, in pre-phase one clinical trials in the context of drug development in collaboration with the pharmaceutical industry. Currently, we are improving the algorithms and are aiming at extending the technique to whole body investigations.

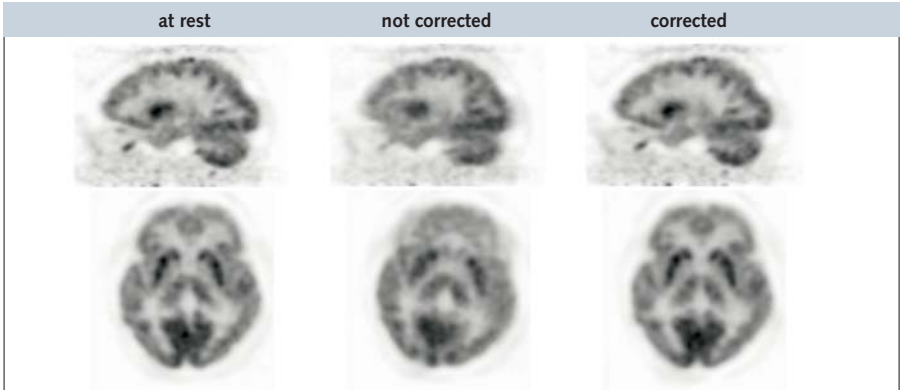

Fig. 4: Images acquired during rest (left) show significantly higher quality than those influenced by patient motion (center). Motion correction (right) essentially restores the full image quality.

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Project partner

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In-beam PET
for radiotherapy monitoring

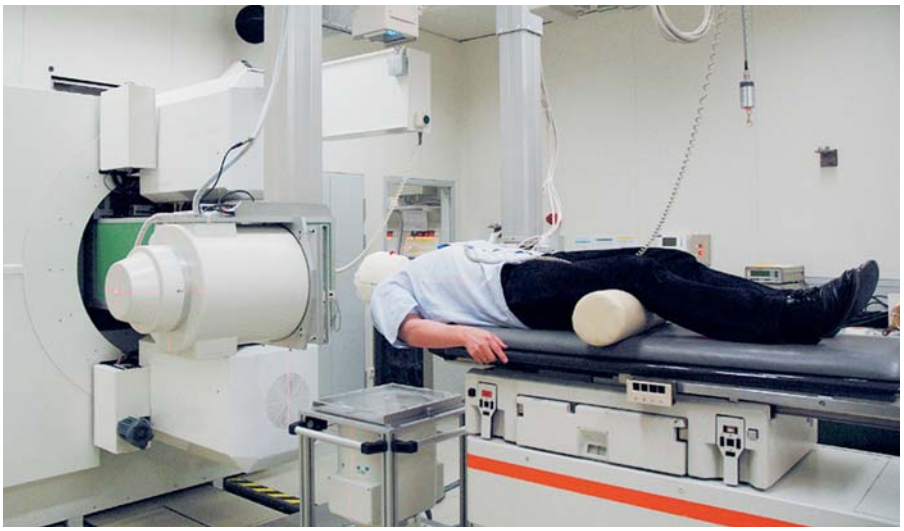

Fig. 1: Double head positron camera developed by FZD at the treatment site of GSI Darmstadt. The horizontal carbon ion beam leaves the beam pipe through a 20 x 20 cm^2 window visible in the centre of the picture. To provide sufficient space for patient positioning, the PET scanner can be moved on rails parallel to the beam between the measuring position displayed and the parking position upbeam. (Photos: GSI)

Wolfgang Enghardt[1], Fine Fiedler, Daniela Möckel, Katia Parodi[2]

Radiation therapy is one of the cornerstones of modern cancer treatment. With increasing tendency, more than 50% of tumor patients are irradiated, either as the exclusive form of treatment or in combination with other modalities such as surgery or chemotherapy. The central challenge of radiotherapy is to destroy the tumor completely while saving the surrounding healthy tissue. In some cases, for example, for compact, deep-seated, radioresistant tumors growing in close vicinity to organs at risk, these objectives cannot be reached by state-of-the-art radiotherapy technology which is based upon hard photon or electron beams delivered by compact electron linear accelerators. Therefore, proton and light ion (e.g., carbon) beams become more and more important due to their favorable physical and radiobiological properties. To translate this potential into clinical results, new technologies in generating, forming, and monitoring ion beams are required. In-beam positron-emission tomography (PET), one of these innovative technologies, has been developed and transferred to clinical application by FZD at the experimental carbon ion therapy facility located at the Gesellschaft für Schwerionenforschung (GSI) Darmstadt in collaboration with the Radiological Clinic of the University Hospital and the German Cancer Research Center Heidelberg.

The unique knowledge of the FZD on in-beam PET is subject of a collaboration with Siemens AG Medical Solutions, with the goal of transferring this into the development of a clinical in-beam PET scanner for ion therapy facilities that are planned or already under construction worldwide. This know-how comprises detector, signal processing, and data acquisition technology for the critical operation of PET detectors at high-energy ion beams [1], tomographic reconstruction algorithms optimized for in-beam PET, and experience in clinical application [2].

Making invisible beams visible

The technological basis of in-beam PET is a double head positron camera [1] integrated into the therapy unit (Fig. 1). During therapeutic irradiation, this device detects the annihilation γ-rays following the decay of minor amounts of positron emitting nuclei (predominantly ^{11}C and ^{15}O) which are produced via nuclear reactions between the impinging ions and the atomic nuclei of the tissue (Fig. 2). Sophisticated algorithms of tomographic reconstruction deliver the spatial distribution of positron emitters *in vivo*. They are related to the dose distribution by means of a precise Monte Carlo simulation of the production of positron emitters and

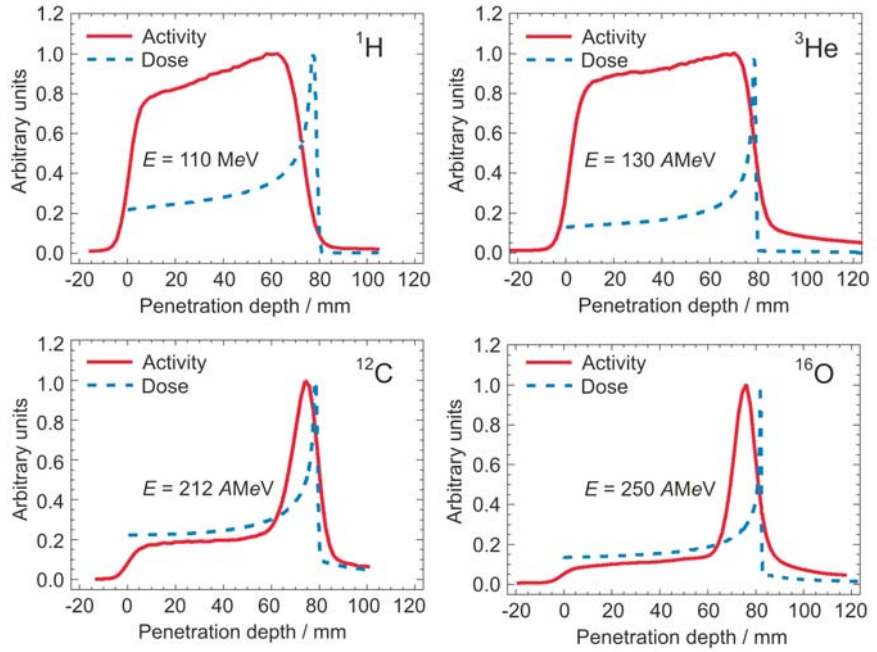

Fig. 2: Depth distributions of calculated dose (blue, dashed) and measured β^+-activity (red, solid) induced by beams of protons as well as ^{3}He, ^{12}C and ^{16}O ions in thick targets of polymethyl methacrylate. The prominent maxima in the cases of ^{12}C and ^{16}O are formed by positron radioactive projectile fragments, whereas the pedestals as well as the distributions generated by ^{1}H and ^{3}He are due to target fragments.

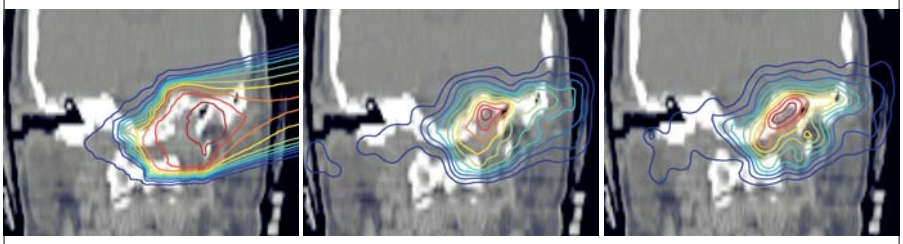

Fig. 3: Clinical application of in-beam PET at the carbon ion therapy facility at GSI Darmstadt. As an example, the irradiation of a chondrosarcoma of the skull base with a lateral portal coming from the left side of the patient, i.e., right side in the picture (maximal dose: 0.63 Gy), is displayed. As indicated by the dose distribution superimposed onto the computed tomogram (left), the carbon ions must not penetrate the brain stem as an organ at risk. The comparison of the predicted (middle) with the measured (right) β^+-activity distributions shows that this was fulfilled during the treatment. The isodose and isoactivity lines are decoded in rainbow colours and denote 5, 15, … 95 % of the maxima.

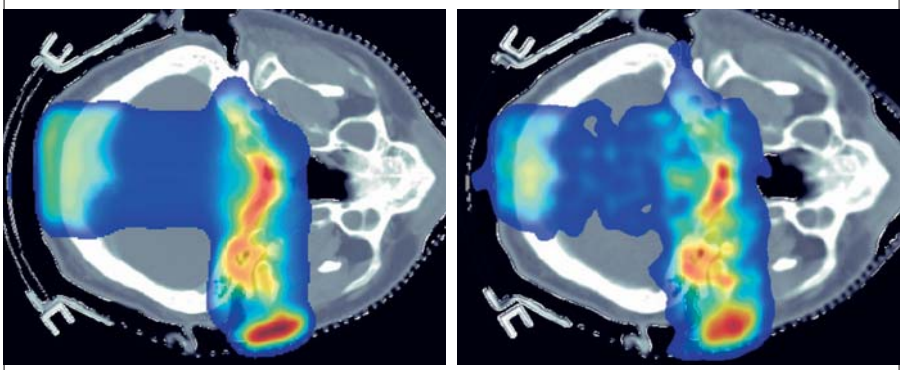

Fig. 4: Monte Carlo calculated (left) and measured (right) activity distribution after proton irradiation of a clivus chordoma patient at Massachusetts General Hospital, Boston. Images by courtesy of K. Parodi and T. Bortfeld.

the detection of annihilation γ-rays (Fig. 3). When these are compared with the measured PET images, deviations between the planned and actual dose distributions can be revealed, quantified [2], and compensated for in the course of further fractionated treatment. Deviations are caused by ion range modifications due to minor patient positioning errors in combination with large tissue density gradients or due to changes of the tissue density distribution within the irradiated volume (e.g., radiation induced tumor shrinking) during the three weeks of fractionated radiotherapy. At the carbon ion therapy facility of GSI, more than 350 cancer patients, most with tumors in the head and neck region, have been treated since 1997 (134 in the years between 2004 and 2006). All of these treatments were monitored by means of in-beam PET for quality assurance. In-beam PET phantom studies with protons at FZD/GSI [3] triggered research on post-radiation PET/CT imaging at Massachusetts General Hospital, Boston (Fig. 4). Initial clinical results [4] confirm the predicted positive impact.

For radiobiological reasons, not only proton and carbon beams are highly desirable for therapy, but also a large variety of ion species with atomic numbers between 1 (hydrogen) and 8 (oxygen). Novel ion therapy accelerators (such as the one at the Heidelberger Ionenstrahl-Therapiezentrum) are capable of delivering beams of all these ions with therapeutically relevant energy values. Since in-beam PET offers the unique possibility to measure particle ranges *in-vivo*, it allows sensitive testing of the physical beam model underlying the dose calculation algorithms for treatment planning. This is highly relevant for commissioning new ion species for therapy. Therefore, the FZD closely collaborating with the Heidelberger Ionenstrahl-Therapiezentrum and the European Organization for Nuclear Research/CERN uses its in-beam PET scanner at the GSI therapy facility to measure physical data which are necessary for extending in-beam PET to other ion beams like helium and oxygen with very high precision (Fig. 2).

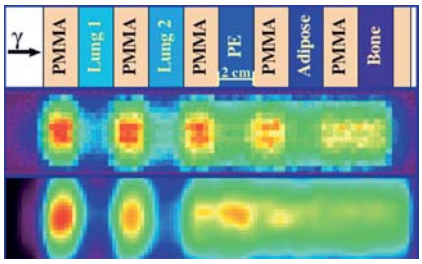

Fig. 5: Two-dimensional β^+-activity distributions generated by ultrahard bremsstrahlung (coming from the left) in an inhomogeneous phantom consisting of 2 cm thick slabs of polymethyl methacrylate (PMMA), polyethylene (PE) and different tissue-equivalent materials (lung, adipose and bone). Upper image: in-beam PET result measured during 34 MV photon irradiation showing the distribution of ^{11}C (half-life: 20 min) and ^{15}O (half-life: 2 min) from the photonuclear reactions $^{12}C(\gamma, n)$ and $^{16}O(\gamma, n)$, respectively. Lower image: result of the PET measurement at the human scanner of the Institute of Radiopharmacy. This measurement started 30 min after finishing the 30 MV photon irradiation. Therefore, it only shows the ^{11}C distribution (^{15}O has nearly completely decayed during 30 min) leading to a reduced contrast between PE, PMMA, adipose tissue, and bone compared to the in-beam image.

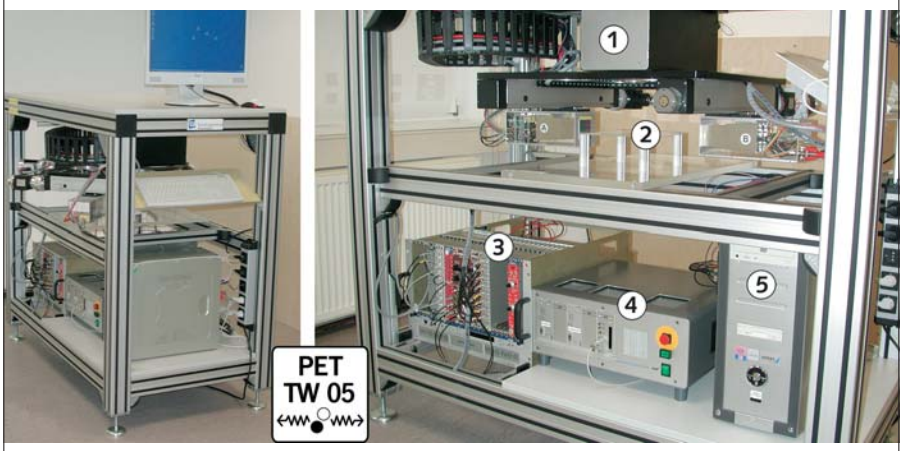

Fig. 6: Left: photograph of the PET-scanner for education. Right: detailed picture with (1) detector system, (2) object table, (3) crate with components for signal processing, (4) step motor controller, (5) standard PC for data acquisition and tomographic reconstruction.

Back to the photons

The successful application of PET for monitoring radiation therapy with ions motivated investigations about the feasibility of the method for hard photon beams. This is studied within the EC FP6 integrated project "BioCare – Molecular Imaging for Biologically Optimized Cancer Therapy" in collaboration with the Karolinska Institute, Stockholm, the Mathematics-Physics Department at Stockholm University, the Soltan Institute for Nuclear Studies, Otwock-Swierk, Poland, and CERN in Geneva. The aim of this research is to prove the feasibility of combining an in-beam PET scanner with an extremely compact 50 MeV electron accelerator delivering a pencil-like beam of ultrahard bremsstrahlung photons. In this case, positron emitters are generated by (γ, n) photonuclear reactions in the tissue at photon energy values above 20 MeV. The in-beam PET-related experimental research of the BioCare project is carried out at 20 until 40 MV bremsstrahlung beams delivered by the Radiation Source ELBE. By means of Monte Carlo calculations, it has already been shown that for electron beam energies beyond 30 MeV, the induced dose-related activity density is comparable with that obtained during irradiation with carbon ions. This has been successfully confirmed in the second step [5] in which the ^{11}C activity generated in plastic phantoms during photon irradiation at ELBE was measured and quantified by means of the human PET scanner of the Institute of Radiopharmacy at FZD (Fig. 5). Just recently, bremsstrahlung-induced positron emitters (^{11}C and ^{15}O) have been imaged in-beam for the first time world-wide by means of a small limited angle positron camera installed at the ELBE beam. The encouraging result was that dosimetry (a collaboration with the Institute of Nuclear and Particle Physics of Technische Universität Dresden) as well as the control of patient positioning on the basis of in-beam PET appears feasible (Fig. 5).

Spin-off for education

Based on the expertise on PET instrumentation available at the Institute of Radiation Physics, a PET scanner has been installed for education (Fig. 6). This device is aimed at practical training in the general principles of tomography, in physics and mathematics of PET imaging and, additionally, in the fundamentals of multi-parameter measurements in nuclear, radiation, and particle physics. The scanner is used at the Institute of Nuclear and Particle Physics of TU Dresden for hands-on training of students of medical radiation sciences at the Postgraduate School of the Center for Radiation Research in Oncology "OncoRay"—located at the Faculty of Medicine of Technische Universität Dresden—as well as of undergraduate physics students.

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Cell damage after X-ray irradiation

Jörg Pawelke, Elke Beyreuther, Wolfgang Wagner

In 1895, Wilhelm Conrad Röntgen discovered X-rays and they were immediately and enthusiastically applied to medical diagnostics. The side effects which were observed, such as skin damage or loss of hair, triggered interest in both the radiobiological properties of X-rays and their use for therapeutic irradiation—the first treatments being performed as early as 1896. More than 100 years after their discovery, X-rays are still the subject of radiobiological research.

In vitro cell experiments allow the study of basic radiobiological effects and mechanisms following an exposure of living cells to ionizing radiation. Such experiments revealed that biological effects in human cells are caused by the fact that the DNA is the main target of radiation. Furthermore, these effects are influenced, among other elements, by the dose and quality of the radiation. The latter is expressed by the quantity of the relative biological effectiveness (RBE), i.e., the ratio of the absorbed doses of two types of radiation producing the same specific effect. While γ-rays were declared to be the reference radiation, an RBE value of 1 was assigned to photon radiations of all energies. However, in vitro studies have already shown that, especially at low doses, low-energy X-rays possess an increased biological effectiveness (RBE > 1) compared to high-energy photons. These differences are interesting in and of themselves, but they must also be taken into account when various photon radiations are used as reference radiation or applied in radiation therapy and diagnostics.

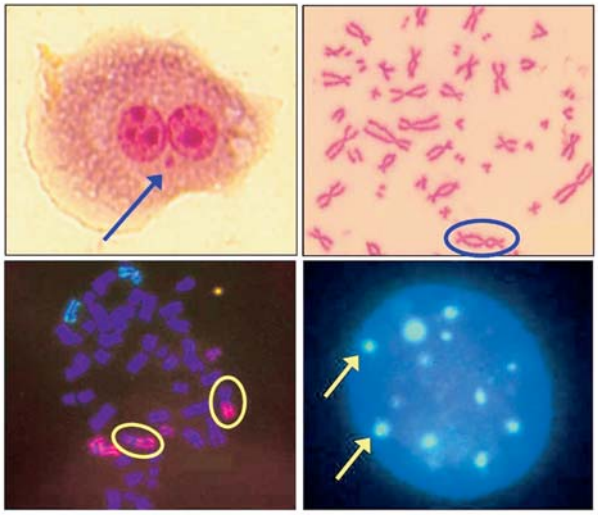


Fig. 1: Mammary epithelial cell damaged by X-ray irradiation with a dose of 2 Gy: chromosomal damage shown by the induced micronuclei (top left), chromosomal aberration (tricentric) determined by fluorescent plus Giemsa staining (top right), chromosome specific aberration (two translocations involving chromosome 1, colored pink) observed by the means of fluorescence in situ hybridization (bottom left) and DNA double strand breaks indicated by the formation of γ-H2AX plus 53BP1 foci (colored green, bottom right).

The study of the RBE dependence on photon energy by means of *in vitro* cell irradiations is part of the radiobiological research at the FZD and subject of collaboration with the Technische Universität Dresden. This interdisciplinary topic necessarily combines the expertise from several fields: (i) operation of an appropriate radiation source, (ii) physical characterization of the radiation field, (iii) irradiation of probes of living cells including an accurate determination of the dose absorbed by the cells, and (iv) suitable methods of determining the radiation-induced biological effects in cells on a cellular and molecular level.

Effectiveness of mammography X-rays

The studies were triggered by an insufficient knowledge of the RBE of low-energy X-rays (below about 30 keV) which are mandatory for mammography because of the necessary tissue contrast. Before establishing nationwide screening programs, the risk-benefit ratio has to be reliably estimated. The relative biological effectiveness determined for various mammalian cell lines ranges from less than one up to about eight and depends on cell line, biological endpoint, and applied reference photon radiation. So far, no experimental studies have been performed on human mammary cells, although these cells require investigation as they are supposed to induce breast cancer.

Therefore, two human mammary epithelial cell lines have been chosen to determine the RBE of 10 kV and 25 kV soft X-rays relative to 200 kV X-rays, all generated by conventional X-ray tubes. Photon energies below 10 keV are less relevant to exposure to the human body due to their strong attenuation in tissue. But they are particularly interesting for biophysical considerations and to show the trend of energy dependence. Various endpoints have been investigated including clonogenic cell survival and several types of DNA damage including the repair kinetics of DNA double strand breaks (Fig. 1). The results [1 – 2] confirm a

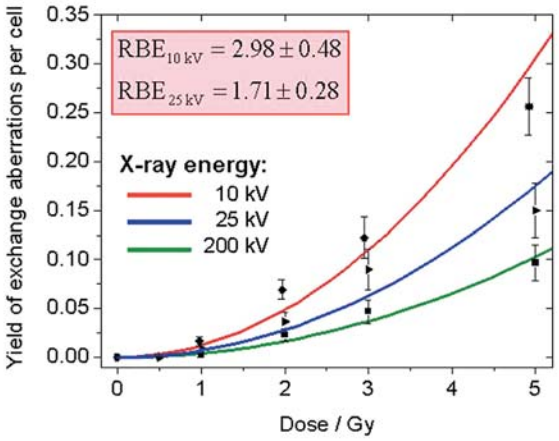


Fig. 2: Yields of chromosomal exchange aberrations per cell induced by different X-ray qualities depending on dose.

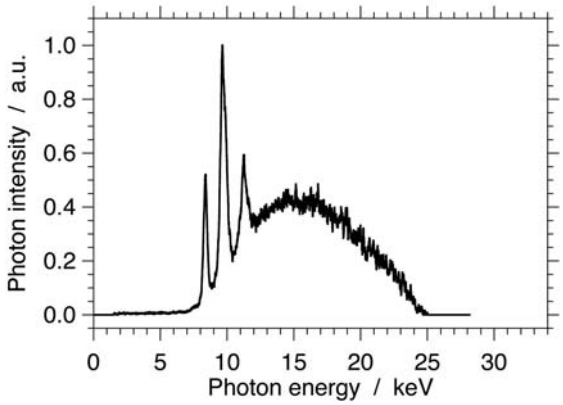


Fig. 3: Energy distribution of an X-ray tube with tungsten anode operated at 25 kV.

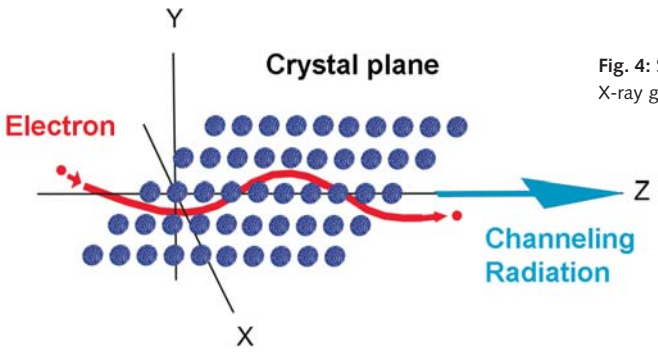


Fig. 4: Scheme of channeling X-ray generation.

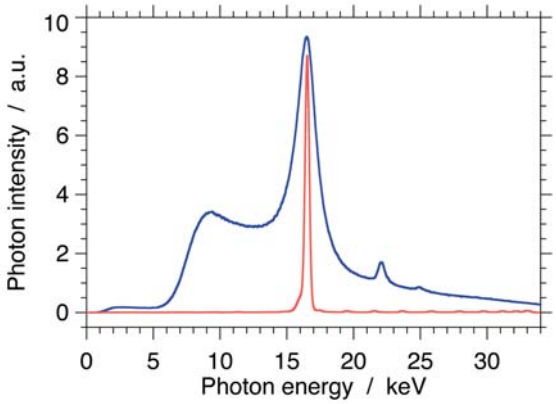


Fig. 5: Spectra of quasi-monochromatic channeling radiation with the associated bremsstrahlung (blue line) measured for 14.6 MeV electrons channeled in the (110) plane of a 42 µm thick diamond crystal. The narrow peak (red line) represents monochromatic X-rays after Bragg reflection at the (002) plane of a planar large-area HOPG crystal.

moderately increased relative biological effectiveness of mammography X-rays ranging between 1 and 2; however, they do not support the high values of a recent claim based on an experiment on cell transformations in a human hybrid cell line and on re-interpretation of various earlier RBE data. Comparable RBE values have been determined for both of the mammary cell lines, displaying the same photon energy dependence (Fig. 2). They are also consistent with experimental data achieved for human lymphocytes as well as with the results of several theoretical calculations of relative biological effectiveness.

Since any material included in the beam can substantially modify the dose absorbed into the cells at these low photon energy values, implementation of an accurate dosimetry [3, 4] considering the exact irradiation conditions was important for the reliability of the data. Thanks to the determination of the X-ray spectra, the contribution of the different energy ranges to the dose could be calculated and compared. Low-energy components are present in the broad X-ray spectrum (Fig. 3), and the biological effects of the different photon energy ranges are superimposed.

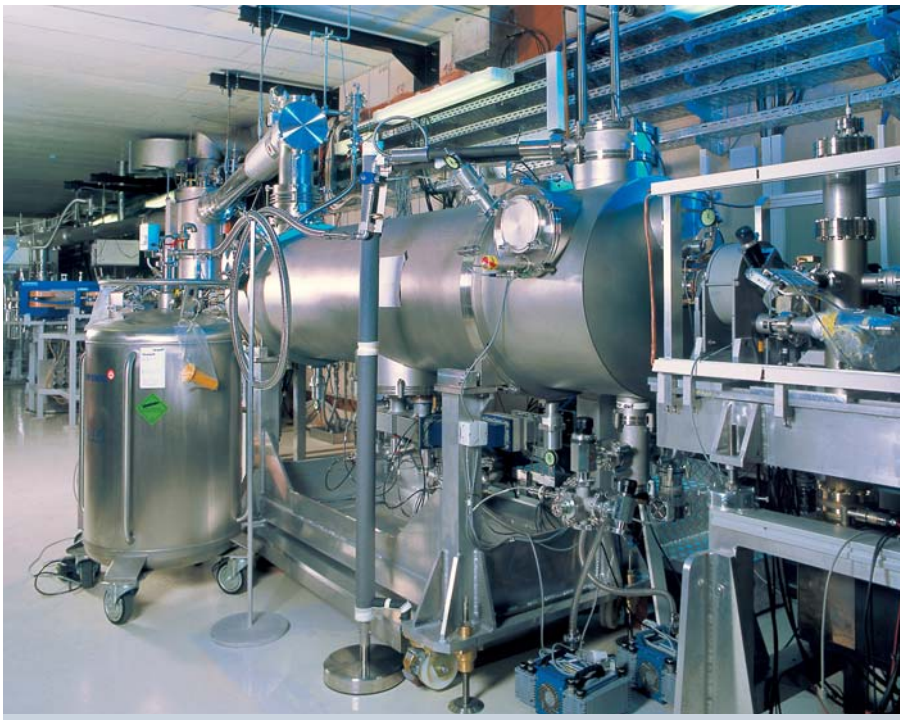
Intense, monochromatic channeling X-rays at ELBE

Experiments with an X-ray tube cannot provide complete photon-energy resolved information on biological effectiveness. Channeling radiation, which is emitted by relativistic electrons during their passage through a diamond crystal parallel to a crystal plane (Fig. 4), was announced as a bright and tunable monochromatic X-ray source in the 1990s. Yet such an unconventional X-ray source, dedicated to practical application, has been realized at the Radiation Source ELBE for the first time.

The yield and spectral distribution of planar channeling radiation have been measured, depending on the crystal properties as well as the parameters of the ELBE electron beam at low beam current (several nA) [5–7]. The results show that by variation of the electron beam energy, the photon

energy can be tuned from 10 keV up to about 100 keV, requiring thicker diamond crystals if large channeling radiation intensities are required. An intense source of channeling radiation dedicated to radiobiological application was designed and implemented at the Radiation Source ELBE. After commissioning, stable operation was proven over hours with an average electron beam current of up to 100 µA, which allows reaching photon rates of quasi-monochromatic channeling radiation of the order of 10^{11} s^{-1}. For this, various technological challenges had to be solved: (i) precise crystal alignment with respect to the beam axis by a newly constructed goniometer, (ii) water-cooling of the crystal while guaranteeing that the electron beam passes through the 150 µm thick diamond crystal of 4 x 4 mm^2 size, (iii) on-line monitoring of the photon-energy spectra at high beam current by means of a Compton spectrometer, and (iv) X-ray monochromatization by applying Bragg diffraction at a highly-ordered pyrolytic graphite (HOPG) crystal. Here, monochromatization means to filter out some part of the spectral distribution at those photon energies where the channeling radiation line superimposes the broad polychromatic bremsstrahlung background naturally associated with the production of channeling radiation (Fig. 5).

Cooperation with industrial partners was essential for the success. The unique HOPG technology of Bourevestnik Inc., St. Petersburg, Russia, allowed designing and building a special toroidal HOPG X-ray reflector which focuses the X-rays on the probe for irradiation of living cells. Radiobiological experiments with quasi-monochromatic channeling radiation X-rays are planned at ELBE at the end of 2007.



Accelerator module of the Radiation Source ELBE. Photo: Sven Claus

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[4]Institute of Applied Problems of Physics, National Academy of Sciences, Yerevan, Armenia

Biomolecular Switches: Molecular evolution conserves function, but allows diversification

Set-up for biophysical experiments with the free-electron laser at ELBE.

Karim Fahmy, Sineej Madathil

The integration of the multitude of biochemical processes occurring simultaneously in a cell critically depends on molecular switching events. They rely on molecules which can adopt different structures, allowing them to interact specifically with other components of a cell. Linking molecular switches into cross-talking signaling chains generates an intricate network of information flow within a cell. Such networks guarantee the proper differentiation, function, and even the initiation of the death of a cell.

G-protein-coupled receptors (GPCRs) form a particularly important class of molecules which switch between functionally active and inactive states after receiving a specific signal. GPCRs couple a large variety of extracellular stimuli to cell-specific answers. Key representatives of GPCRs are neurotransmitters and hormone receptors. Upon ligand binding at the extracellular side of the receptors, these membrane proteins alter their structure at the intracellular side. This allows interaction with guanosine-binding proteins (G-proteins) which initiate signal amplification and transduction within the cell. The human genome contains about 1000 GPCRs. Each member of this receptor superfamily is a potential drug target and approximately 50% of today's pharmacotherapeutics target at GPCRs. Intensive structural and functional studies try to exploit their pharmacological potential.

Within evolutionary related GPCRs, functional mechanisms are believed to be conserved; however, it is not understood how an extremely large variety of

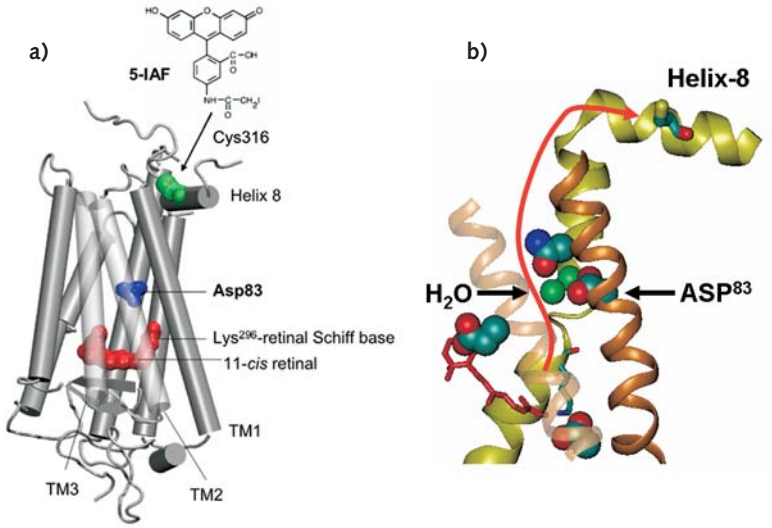

Fig. 1: a) Sketch of the rhodopsin structure (pdb id 1L9H) emphasizing the location of the covalently bound fluorescent dye fluorescein at helix 8 and the position of protonated Asp^{83}, for which the C=O stretching mode has been assigned and used here to monitor changes in the intramembrane H-bond network above the 11-*cis*-retinal. TM1 – 3 refers to the intramembraneous helices 1 – 3, whereas helix 8 runs roughly parallel to the cytosolic membrane surface. **b)** Fluorescence-infrared-cross-correlation spectroscopy evidences the kinetic coupling between the physical environment of helix 8 and the intramolecular H-bond network connecting Asp^{83} to the conserved NPxxY motif through two water molecules (green). This provides a signaling pathway from the interior of the GPCR to its cytosolic surface as indicated by the red arrow.

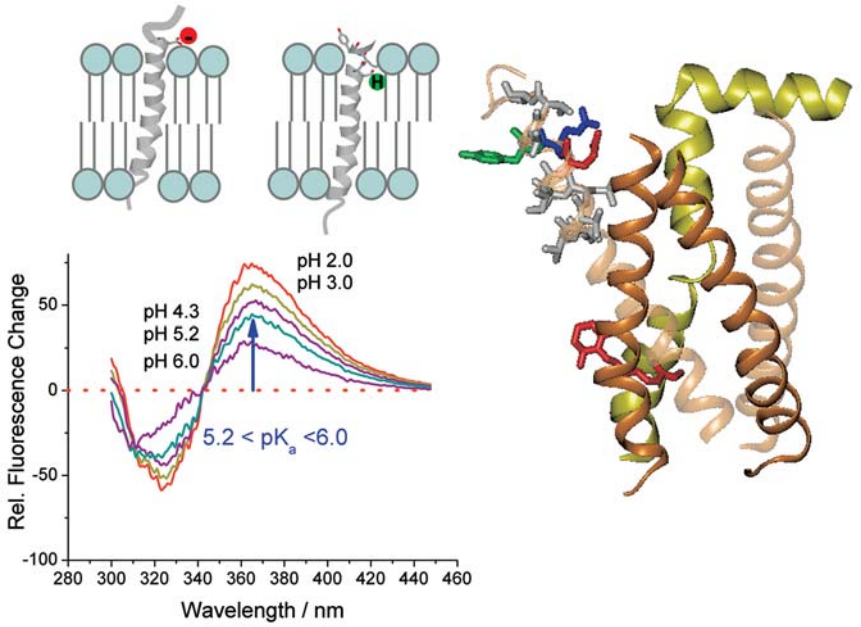

Fig. 2: Fluorescence spectroscopy of an isolated synthetic hydrophobic peptide corresponding to helix 3 of rhodopsin exhibits proton-sensitive fluorescence energy transfer from the native tyrosin in the conserved ERY motif to an engineered tryptophan. The unusually high pK of ~6 is assigned to the neighboring strictly conserved glutamic acid as the only titratable group in this pH range [5]. The high pK indicates that the protonated (neutral) state of the Glu^{134} side chain becomes stabilized by hydrophobic interactions in a surrounding lipophilic phase as presented in the bilayer model above. The right panel shows the position of the ERY motif in the context of the full-length receptor, where it localizes close to the membrane water interface.

structurally different ligands can operate essentially the same mechanism. We have studied the physical basis of switching in the bovine photoreceptor rhodopsin, the only GPCR for which X-ray structures have been determined, so that structural models of receptor activation can be generated [1]. Rhodopsin provides the molecular basis of vision. It is a prototypical GPCR which allows transducing the absorption of light into a nerve pulse in the retina of the eye. The process of vision is primarily based on the photoisomerization of the covalently bound ligand 11-*cis* retinal, which evokes structural changes in rhodopsin very similar to the changes induced by the binding of a hormone to a hormone receptor. Today, most studies of rhodopsin aim at an understanding of general switching mechanisms in GPCRs.

The plasmamembrane: A specific reaction environment involved in conformational switching of GPCRs

Our work focuses on the role of specific lipid protein interactions within the plasma membrane which affect the switching process of GPCRs. The function of the lipid bilayer into which GPCRs are integrated is not deducible from the crystal structures, where lipids are absent. Using spectroscopic techniques, we have investigated two regions of rhodopsin which are involved in functionally important lipid protein interactions. One region is the C-terminal extramembraneous amphipathic helix 8. In photoactivated rhodopsin, it binds to the G-protein. It has been suggested to be a "local" switch region because it can adopt different structures even in the absence of the rest of the receptor molecule, depending on the surrounding lipids [2].

To understand the long-range coupling of the cytosolic helix 8 to the transmembrane region where the light-dependent structural alteration occurs, we have simultaneously observed two independent spectroscopic properties by which

structural transitions can be monitored: fluorescence emission and IR-absorption. In cooperation with Ulrike Alexiev from Freie Universität Berlin, this experiment has allowed us to identify highly synchronized changes around the lipid-interacting helix 8 (where a fluorescent label was attached to Cys^{316}) and the transmembrane H-bond network around Asp^{83} in the interior of the protein. The latter was monitored by infrared spectroscopy. The data reveal a structural connectivity that couples photoisomerization to a lipid-dependent switching motif at the extramembraneous G-protein recognition site [3]. In contrast, another internal domain around Glu^{122} communicates with an H-bond network which is not coupled to the helix 8 environment, but appears to be important for retinal binding.

The kinetic analysis of distant conformational changes within rhodopsin clearly shows that ligand specificity can evolve independently from evolutionary conservation of function. This is strongly supported by the fact that Asp^{83} and the transmembrane motif to which it is linked through water molecules have been strongly conserved throughout evolution, comprising many hormone receptors, whereas Glu^{122} has only been conserved in the subfamily of photoreceptors.

Hydrophobicity and side chain protonation in GPCR function: Searching for artificial mimics of natural switches

We have extended our studies to another surface region of rhodopsin which also interacts with the lipidic matrix and have started to systematically explore the role of side chain hydrophobicity in membrane proteins. Hydrophobicity describes the ease by which a chemical group can be transferred from an aqueous to a nonpolar environment such as the interior of a plasma membrane. Recently, the biological importance of hydrophobicity for the insertion of membrane proteins into the plasma membrane has been accurately quantified. This led to a scale of free energy of the transfer of any of the 24 naturally-occurring amino acids in proteins from a hydrophobic to an aqueous environment [4]. Importantly, the influence of the charges of a protonatable group in these amino acids has been addressed. We have investigated whether the modulation of side chain hydrophobicity by protonation may provide a link between proton exchange reactions occurring in GPCRs and other membrane proteins and the transition between their functionally distinct states. Surprisingly, a specific single transmembrane-spanning domain of rhodopsin, i.e., one helical rod-like structural element out of seven which make up a GPCR, exhibits proton-dependent structural changes in an artificial membrane [5].

In cooperation with the Max Planck Institute of Molecular Physiology, Dortmund, and M-fold GmbH, Tübingen in a project funded by the Federal Ministry of Education and Research (BMBF), we are applying our approach to a large scale of synthetic peptides with charged residues strategically located near the water lipid phase boundary, where proton-dependent structural reorganizations can be expected. These model systems will not only verify important hypotheses on GPCR function but the knowledge gained from this class of membrane receptors will also allow the synthesis of artificial transmembrane switching devices such as the trans-membrane proton sensor which we are currently developing in this project.

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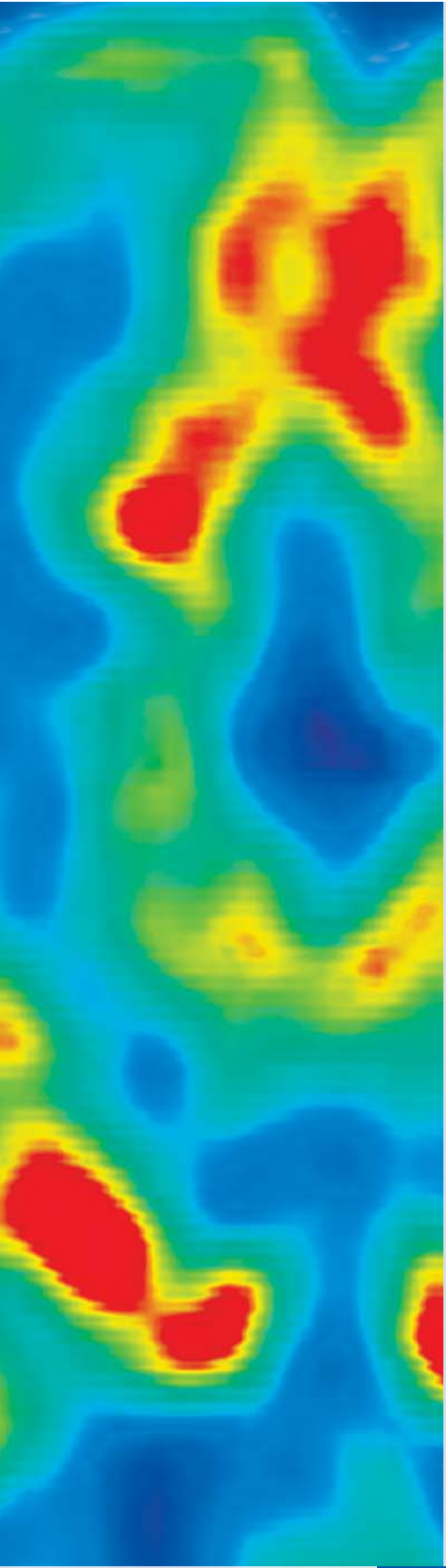
Project partners

[1] Indian Institute of Technology Madras (IITM, Chennai)

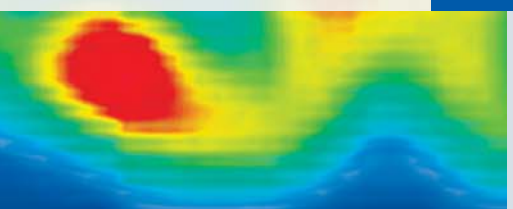
[2] Freie Universität Berlin, Germany



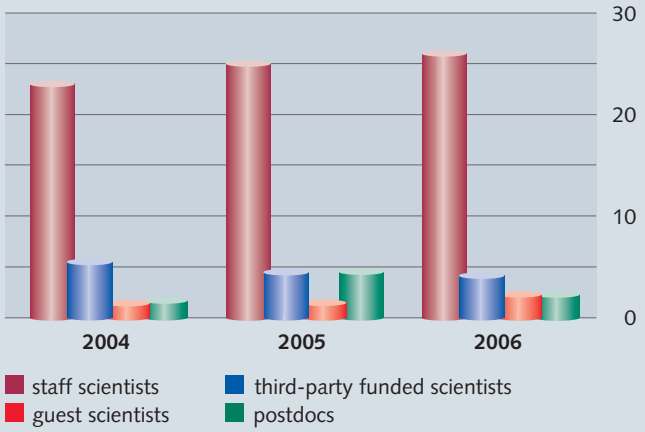
The Forschungszentrum Dresden-Rossendorf (FZD) is a multi-disciplinary research center for natural sciences and technology. It is the largest institute of the Leibniz Association and is equally funded by the Federal Republic of Germany and the Federal States, in particular by the Free State of Saxony. At the FZD, around 225 scientists are engaged in three different research programs of basic and application-oriented research. Scientists working in the Structure of Matter program investigate the reactions of matter when influenced by high fields and minuscule dimensions. Research and development in the Life Sciences program is focused on the imaging of tumors and the effective radiation treatment of cancer. How can humankind and the environment be protected from technical risks? – This question is in the center of research in the Environment and Safety program of the FZD.

In the following Facts & Figures section data presenting the scientific output in the Life Sciences research program are given as well as information on staff and funding at the FZD.

Facts & Figures



Scientific staff

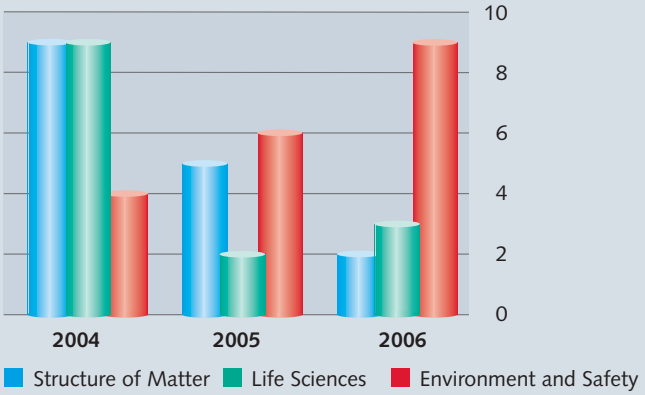


This chart shows the evolution of posts occupied by scientific personnel in the Life Sciences program of the FZD. Third-party funded scientists, guest scientists, and postdocs represented by the corresponding figures are given in units of paid full-time posts.

Budget	2004		2005		2006	
Research Programs	Public Funding T€	Project Funding T€	Public Funding T€	Project Funding T€	Public Funding T€	Project Funding T€
Structure of Matter	13.858	1.190	14.209	1.259	19.575	1.405
Life Sciences	**8.241**	**801**	**6.266**	**645**	**8.191**	**784**
Environment and Safety	13.005	3.180	12.950	3.656	11.866	3.990
Facilities	20.721	911	20.428	756	17.988	1.643
Sum	55.825	6.082	53.853	6.316	57.620	7.822

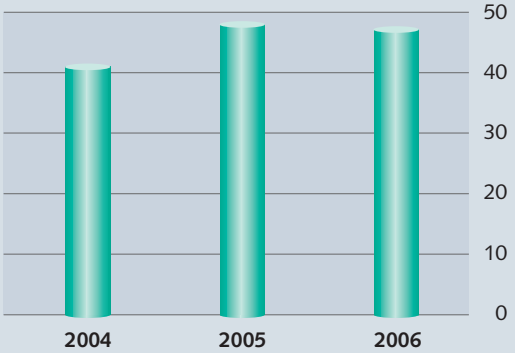
This table displays the share of each research program as well as the experimental facilities located at the FZD of both public and project funding during the last three years.

Patents



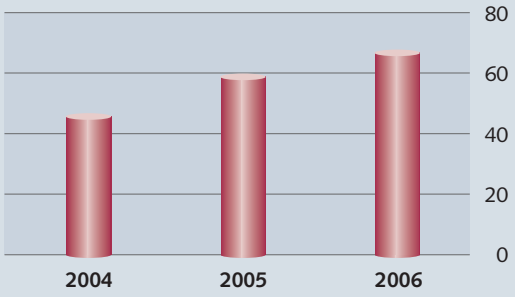
This figure shows the number of applications for a patent filed in each research program of the FZD during the last years. National and international applications for a patent of one and the same invention are only counted once.

Publications



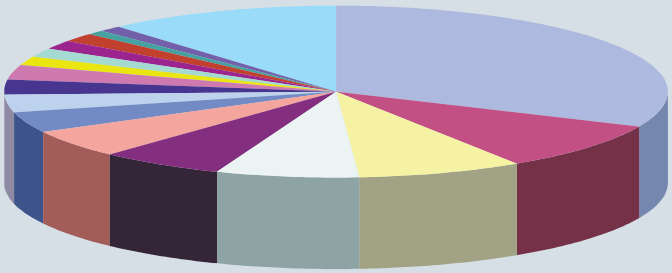
This chart displays the evolution of peer-reviewed articles by scientists from the FZD's Life Sciences program.

Doctoral students



This figure shows the evolution of the doctoral students at the FZD from 2004 until 2006.

International guest scientists



Russia	99	Latvia	13	Spain	5
Ukraine	29	India	10	USA	5
Bulgaria	25	Hungary	9	Romania	3
Czech Republic	22	Algeria	8	Australia	3
Poland	19	Japan	6	other	36
China	16	Israel	5		

Here, the distribution of the international guest scientists who visited the FZD for the purpose of research between 2004 and 2006 is shown according to their countries of origin.

Organizational Chart

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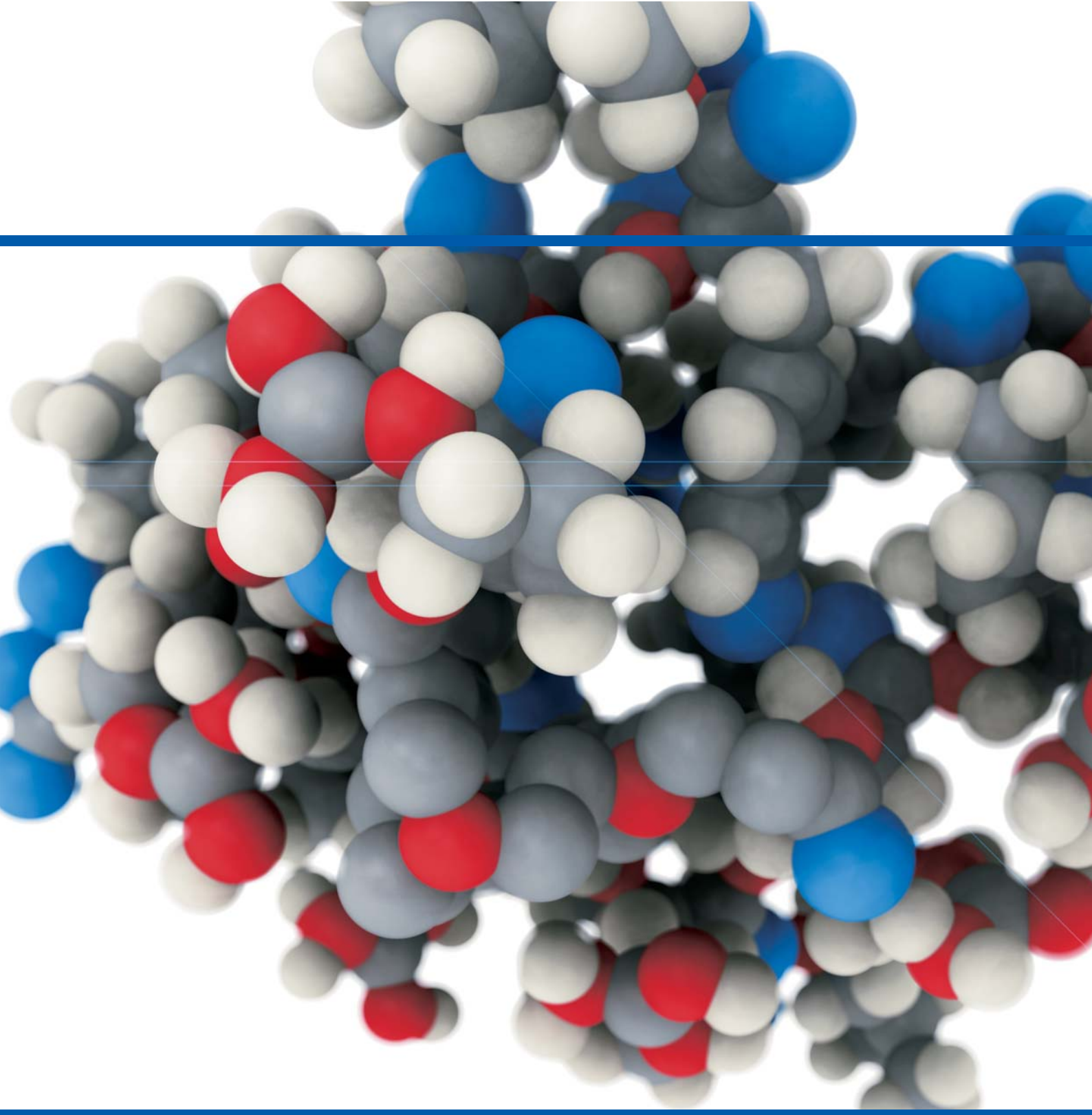

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