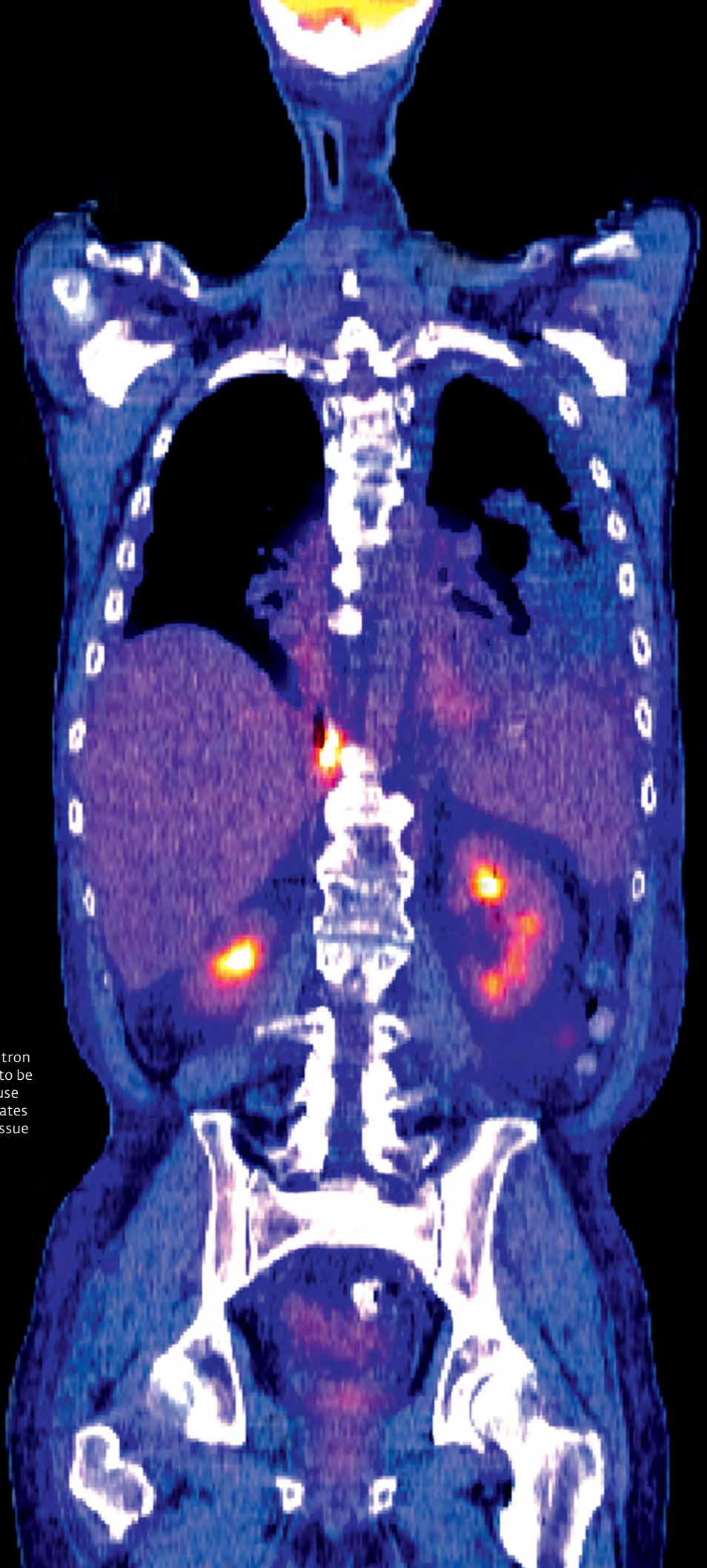


Widespread in cancer diagnostics: Positron emission tomography enables tumors to be identified, like this one in a lung, because the glucose in it, for instance, accumulates fluorine-18. On the images, diseased tissue is clearly recognizable as a bright area.

The Stuff of **Enlightening Diagnoses**

Doctors today already frequently rely on positron emission tomography – PET for short – in cancer diagnostics. However, in order to use this method for other diseases, too, they need suitable tracer substances containing radioactive fluorine-18 – a challenge for **Tobias Ritter** and his team at the **Max Planck Institut für Kohlenforschung** in Mülheim an der Ruhr. The chemists are searching for ways to label diverse molecules with fluorine-18 and thus expand the range of possibilities for medical specialists.

TEXT **KARL HÜBNER**

Every couple of days, early in the morning, a messenger pulls up to Kaiser-Wilhelm-Platz 1 in southern Mülheim to deliver a small, rather unspectacular stainless-steel box. Each time, two employees of the Max Planck Institut für Kohlenforschung are already waiting at the entrance to the building to accept the delivery. A couple of signatures, then straight to the lab they go with the box. The heavy security door and the sign with the familiar international radiation symbol make it quite clear: radioactive substances are used in this lab.

The box that was just delivered also contains such a substance: fluorine-18. Unlike natural fluorine, which has a mass number of 19, fluorine-18 is synthetically produced and is highly unstable. Its half-life is 110 minutes, then it decays into oxygen-18. When that happens, high-energy radiation is released, which is why the chemists in Mülheim use a special lab when they work with fluorine-18. And they have to work fast: after 110 minutes, half of the fluorine atoms have already disappeared; after 220 minutes, three-quarters of them; and so on.

MOLECULES FOR INNOVATIVE DIAGNOSTIC APPLICATIONS

The researchers are pursuing a specific goal with their work against the clock. "We are looking for ways to incorpo-

Photo: Science Photo Library/LIVING ART ENTERPRISES, LLC

rate fluorine-18 into molecules that permit novel diagnostic applications of positron emission tomography," explains Tobias Ritter. A chemist, he has been Director of the Organic Synthesis department at the Max Planck Institute in Mülheim since 2015. Fluorine chemistry and especially the short-lived fluorine-18 are key topics of interest for his group. Positron emission tomography, a well-established method for tumor diagnostics, relies on radioactive substances that release positrons. The special radiochemical laboratory was set up a year ago specifically for the purpose of conducting synthesis experiments with these positron suppliers.

HOW EASY IS IT TO FLUORINATE SUBSTANCES?

At the heart of the lab are two chambers surrounded by lead walls and a thick leaded glass window. In technical jargon, shielded chambers like these are called hot cells. It is into just such a chamber that the researchers in Mülheim now place the metallic cylinder they liberated from the freshly delivered stainless steel box. It's barely larger than a normal tin can, but it weighs 15 kilograms. "Solid lead," explains Matthew Tredwell, who heads the radiochemical lab in Ritter's department. Only when the hot cell has been completely sealed off again does one of his colleagues remove the cylinder's lid. This requires highly focused concentration as she operates the external controls that steer the steel gripper arms protruding into the sealed chamber.

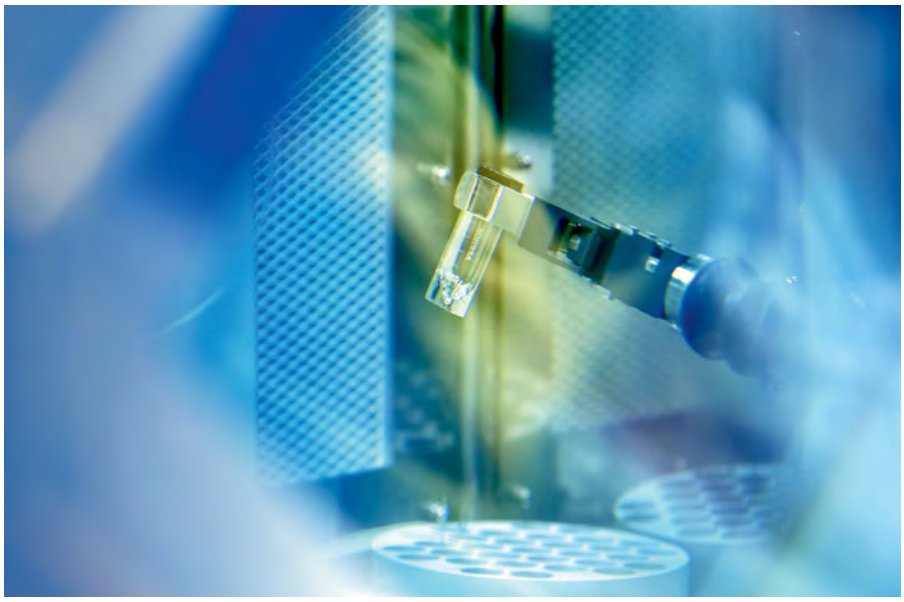
Then she removes the cargo's key component: a small vial containing a clear liquid. "It's simply water in which fluoride-18 ions have been dissolved," Tredwell explains. And as if the few milliliters of liquid didn't already appear tiny enough given their 15-kilogram packaging, the chemist adds that the amount of fluorine in the liquid is just over one picogram – one trillionth of a gram.

Despite being an inconceivably small amount, it is plenty for the experiments the scientists have planned for this day. They want to test how easily they can fluorinate other substances – that is, incorporate a fluorine atom into them. The working group already produced the necessary synthesis components previously, and they are now waiting in small vials in the hot cell. The rest happens automatically: a sort of hollow needle goes to each vial, withdraws a precisely determined quantity and combines the desired reactants. To be able to then immediately examine whether and in what amount and purity the desired products were formed, the radiochemical lab also contains analytical equipment. These devices have recently repeatedly shown that the researchers are on the right track.

THE METHOD HAS GREAT POTENTIAL

Verena Ruhlmann, a physician, finds it very interesting, too. She was in attendance when the Max Planck Institute presented its special laboratory to the residents of Mülheim in late 2016. In fact, she was invited to be a speak-

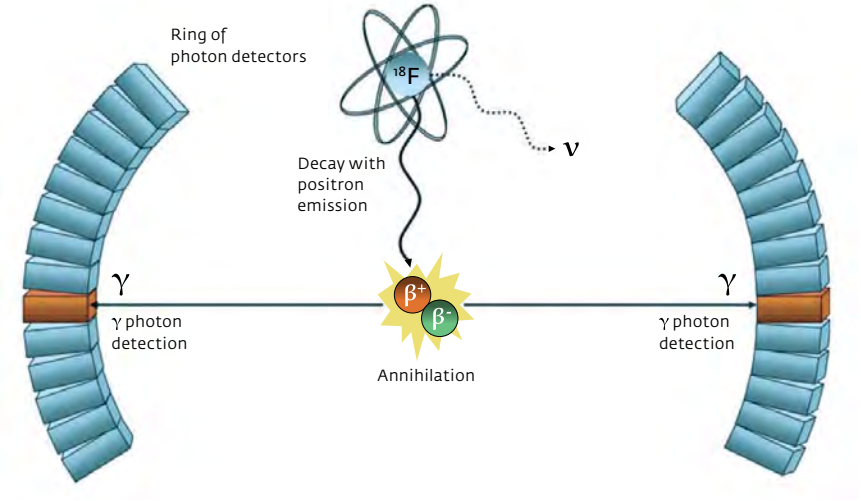
Photo: Frank Vinken



Secure access: In a special lab, Marta Brambilla (left) controls a gripper arm that protrudes into a chamber for radiochemical experiments. This hot cell has lead walls and leaded glass windows to shield the radioactive radiation. The chemists place all the components for their planned reactions in there in small vials (right) – including substances that contain radioactive fluorine-18. A robot then mixes the source materials for the reactions together.

er that day. This is surprising at first, since Ruhlmann has no connection with the Max Planck Institute – her workplace is actually several kilometers away to the east. In the neighboring city of Essen, she is a senior physician in the Essen University Hospital Clinic for Nuclear Medicine. That evening, she spoke about what positron emission tomography is used for at her clinic. One area is tumor diagnosis, where PET is used, for example, to distinguish between benign and malignant growths. Or to visualize changes in lymph nodes. Or to show whether and how well a treatment is working. And also whether a tumor may have returned after an initial successful treatment. Ruhlmann raved about what an "outstanding method" it is. Her department already uses PET to examine around 25 patients every day. But the physician also emphasized that she sees even greater potential for PET in the future, both in tumor diagnostics and for other diseases, such as Alzheimer's, Parkinson's and cardiovascular disorders.

It is this vision that unites facilities like Essen University Hospital and the Max Planck Institut für Kohlenforschung. After all, the new substances that Ritter's team is attempting to manufacture in the radiochemical lab could one day also be of interest to nuclear medicine specialists like Verena Ruhlmann. >



Indirect detection: Beta decay of fluorine-18 releases a positron (β+), the antiparticle of the electron, and a neutrino (ν). The positron immediately annihilates with an electron (β-), emitting two γ-photons in precisely opposing directions. These are then detected, making it possible to reconstruct where the fluorine-18 decayed.

Photo: Frank Vinken; graphic: Angew. Chem. Int. Ed. 2008, 47, 8998 – 9033

As she explains, “For every PET scan we perform, we need, of course, a substance that releases positrons.” Fluorine-18 is by far the most important isotope for PET tracers – what experts call the substances whose path in the body can be traced during a scan.

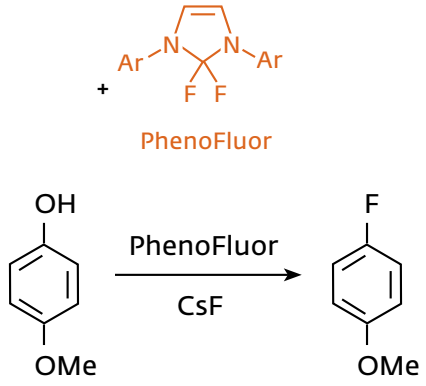
For most PET scans, however, simply administering fluorine-18 in the form of a fluoride salt would be pointless. At most, it would accumulate in the bones, but not where it is needed for most diagnostic tasks. For that reason, chemists are attempting to incorporate the fluorine into molecules that are suitable for use as tracers. These are molecules that accumulate as selectively as possible – that is, exclusively – at the sites in the body that are relevant for the diagnosis. Those sites then become visible from outside the body.

TRACERS THAT DOCK ONTO PLAQUE IN THE BRAIN

Currently, the most important such molecule is a fluorodeoxyglucose. This substance is similar to normal glucose; the only difference is that one carbon atom carries a fluorine atom – a fluorine-18. Despite this modification, in the body, the fluorinated sugar behaves almost like glucose. Therefore, once it is injected into the bloodstream, this substance goes wherever

Pioneering new PET applications: Tobias Ritter and his team develop tools in order to produce new tracer substances for diagnosing such things as tumors and cardiovascular diseases.

Photo: Frank Vinken



Key reaction: Tobias Ritter found a substance – PhenoFluor – that can be used to specifically replace OH groups with fluorine atoms.

there is a demand for sugar. Demand is particularly high in most tumor cells. Fluorodeoxyglucose thus makes tumors easily visible in a PET scan.

At Essen University Hospital, Verena Ruhlmann also has access to a radiochemical lab; there, this substance is manufactured fresh every day because the radioactive isotope decays too quickly to be stored for long periods. For patients, though, the particularly short lifespan of the PET tracers is an advantage, as it means that the already low radioactivity in their body completely subsides relatively quickly. Contributing to this is the fact that fluorine-18, like other tracer substances, is transformed exclusively into stable isotopes and leaves no radioactive derivatives behind.

If the PET visions are to become reality, then many other PET tracer molecules besides the fluorinated sugar will be needed. Molecules, for instance, that dock onto the brain plaque that is characteristic for certain forms of dementia – an initial Alzheimer's tracer is already on the market. Or molecules that, in tumor diagnostics, would enable predictions as to which cancer drug is best for an individual patient.

FLUORINE IS EXTREMELY REACTIVE

In order for them to reach their targets in the body, the molecules must have a suitable chemical structure. However, to function as tracers, they must also include, for example, fluorine-18. And that's precisely where the problem – and the work of the chemists in Mülheim – begins.

Incorporating fluorine into a more complex organic molecule isn't always as easy as synthesizing fluorodeoxyglucose, which can be done with a simple fluoride solution. Normally, considerable thought must be given as to how to source even natural fluorine-19. "Elemental fluorine is generally out of the question, as it is much too reactive," explains Tobias Ritter. Fluorine is more reactive than any other element, so if pure fluorine were used for fluorination, it would, in most cases, affect multiple locations in the molecule rather than selectively affect just the one desired location.

On top of this, elemental fluorine is extremely difficult to handle in the lab. In compounds, on the other hand, it is often bound so stably that it requires an enormous amount of energy to activate it. This property played no small part in helping fluorochlorocarbons and also Teflon®, a fluorochemical, make it big. >

Checking the result: Matthew Tredwell uses high-performance liquid chromatography, or HPLC, to analyze whether a reaction produced the desired products.

Photo: Frank Vinken

Radiation-proof construction: Steel plates on which lead slabs rest are mounted on stable beams in the ceiling of the special radiochemical lab (top). The walls are built from radiation-protection bricks; their dark coloring is due to the large amounts of iron oxide they contain (bottom left). Iron oxide particles are added to the mortar (bottom right).

In the case of fluorine-18, its short lifespan is an additional complicating factor, forcing Tobias Ritter and his colleagues to completely rethink their approach. "In the case of complex target molecules, a chemist would normally try to incorporate the fluorine in as early a reaction step as possible," Ritter explains, "because the molecule becomes ever more complex with each additional step. This, in turn, increases the risk that the fluorination will no longer be selective, but will occur at various sites in the molecule."

With volatile fluorine-18, however, it isn't possible to fluorinate in an early stage of synthesis. A complex molecule is frequently constructed over the course of ten or even more steps, each of which takes some time. If the radioactive fluorine were to be incorporated in an early phase, there would be hardly any of it left in the finished molecule. "So with fluorine-18, we have to find a way to incorporate it only in the last or perhaps next to last reaction step," says Ritter, and immediately adds: "That's precisely what is so difficult."

OH GROUPS CAN BE SPECIFICALLY REPLACED

In 2011, though, the scientific community sat up and took notice. In a scientific journal, Ritter, who at the time was still conducting research at Harvard University in Cambridge, Massachusetts, and two of his colleagues published a method by which OH groups can be specifically replaced by fluorine atoms in a certain class of molecules. At the heart of the method was a reagent that was later given the commercial name PhenoFluor. The researchers found that it could be used to fluorinate a number of even quite complex organic molecules with good yields. There was just one requirement: the initial substance had to carry an OH group at the site where the fluorine was to end up. A milestone.

But there was one thing that didn't work even with PhenoFluor: the incorporation of fluorine-18. The problem

Photos: M. Teske

» We expanded the set of chemical building blocks with which we can produce fluorine tracers for PET.

was that PhenoFluor contains two fluorine atoms that are so homogeneous that it isn't possible to control which of the two ultimately carries out the actual fluorination step. "We would have needed to create a PhenoFluor with two F-18 atoms, but that isn't possible for technical reasons because fluorine-18 is always contaminated with fluorine-19," explains Tobias Ritter. They therefore needed a different reagent.

Another five years passed before the researchers solved this problem. Their trick was to first use a reagent that has a similar structure to PhenoFluor but that contains, in place of the two fluorine atoms, two atoms of chlorine, a chemical relative. This substance allows a similar reaction to be carried out as with PhenoFluor, but the chemists intervene again before its final step. "In that moment, one of the chlorine atoms is present as a chloride ion, which we then replace with a fluoride-18 ion using a common technique," says Ritter. This ensures that the OH group is exchanged for the fluorine-18.

"With this reaction, we expanded the set of chemical building blocks with which we can produce fluorine tracers for PET," says Ritter. But that won't happen overnight. In many cases, it isn't yet known exactly which molecules medical diagnostics will ultimately really need. To more closely align the chemical tools with medical demand, Ritter's department is collaborating with clinical facilities such as Essen University Hospital and also Massachusetts General Hospital in Boston. Their goal is to identify suitable molecules for very specific fields of application and to develop feasible methods for synthesizing them. Then, in the second step, fluorine-18 has to be integrated into these molecules in order for them to function as PET tracers.

It isn't yet possible to say whether the existing fluorination tools will suffice for this, as it depends on the individual target molecule. In any case, the researchers are working in their radiochemical lab to create more tools for syntheses with fluorine-18. After all, they want to expand the spectrum of fluorinatable molecule classes. So the messenger will continue to make regular trips to Kaiser-Wilhelm-Platz to unload the 15-kilogram metal case with the trillionth of a gram of fluorine-18. ◂

TO THE POINT

- **Positron emission tomography requires tracer molecules with radioactive atoms, such as fluorine-18. However, it is difficult to synthesize such molecules due to the short lifespan of these atoms.**
- **Chemists working with Max Planck Director Tobias Ritter are developing methods to specifically attach fluorine-18 to desired sites in a molecule. They conduct the corresponding experiments in a special radiochemical lab.**
- **Initial success: a synthesis method that replaces OH groups with fluorine-18 in many organic molecules, including in complex ones. In the future, the Max Planck researchers hope to further optimize this method and extend it to even more complex molecule classes.**

GLOSSARY

Half-life: The time after which the amount of a radioactive isotope has been halved. Every radioactive isotope has a constant rate of decay – and thus a fixed and unambiguous half-life.

Isotope: Chemical elements can occur in different atomic versions due to a difference in the number of neutrons in the atomic nucleus. The different versions of one and the same element are known as isotopes.

Positron: An elementary particle that is also considered to be an antiparticle. It is the same size and weight as an electron, but has the opposite electrical charge – that is, a positive one. A positron annihilates with an electron, releasing energy in the form of two gamma quanta. Since all matter contains electrons, the annihilation occurs practically immediately, so positrons always exist only briefly.

Positron emission tomography (PET): An imaging technique that, like magnetic resonance tomography (MRT) or computer tomography (CT), is capable of producing sectional images of individual layers inside the body. The method requires substances containing the radioactive elements fluorine-18 or gallium-68, which accumulate for instance in tumors and emit positrons as they decay. These elementary particles immediately annihilate with an electron – essentially at the site where they come into existence – releasing two radiation pulses in precisely opposing directions. If detectors register these pulses outside the body, it is possible to calculate the precise location where the fluorine atom decayed.