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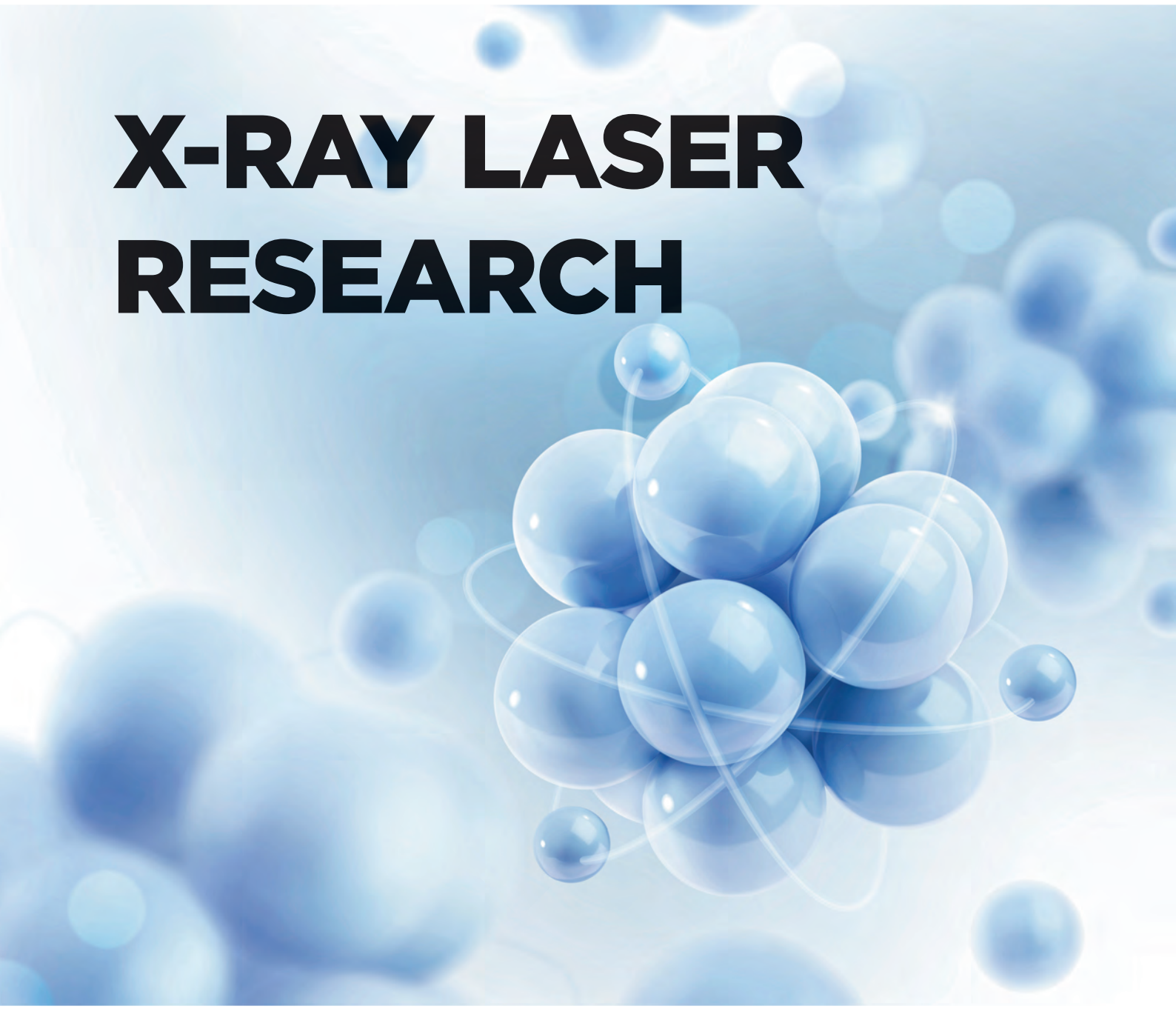
X-RAY LASER RESEARCH



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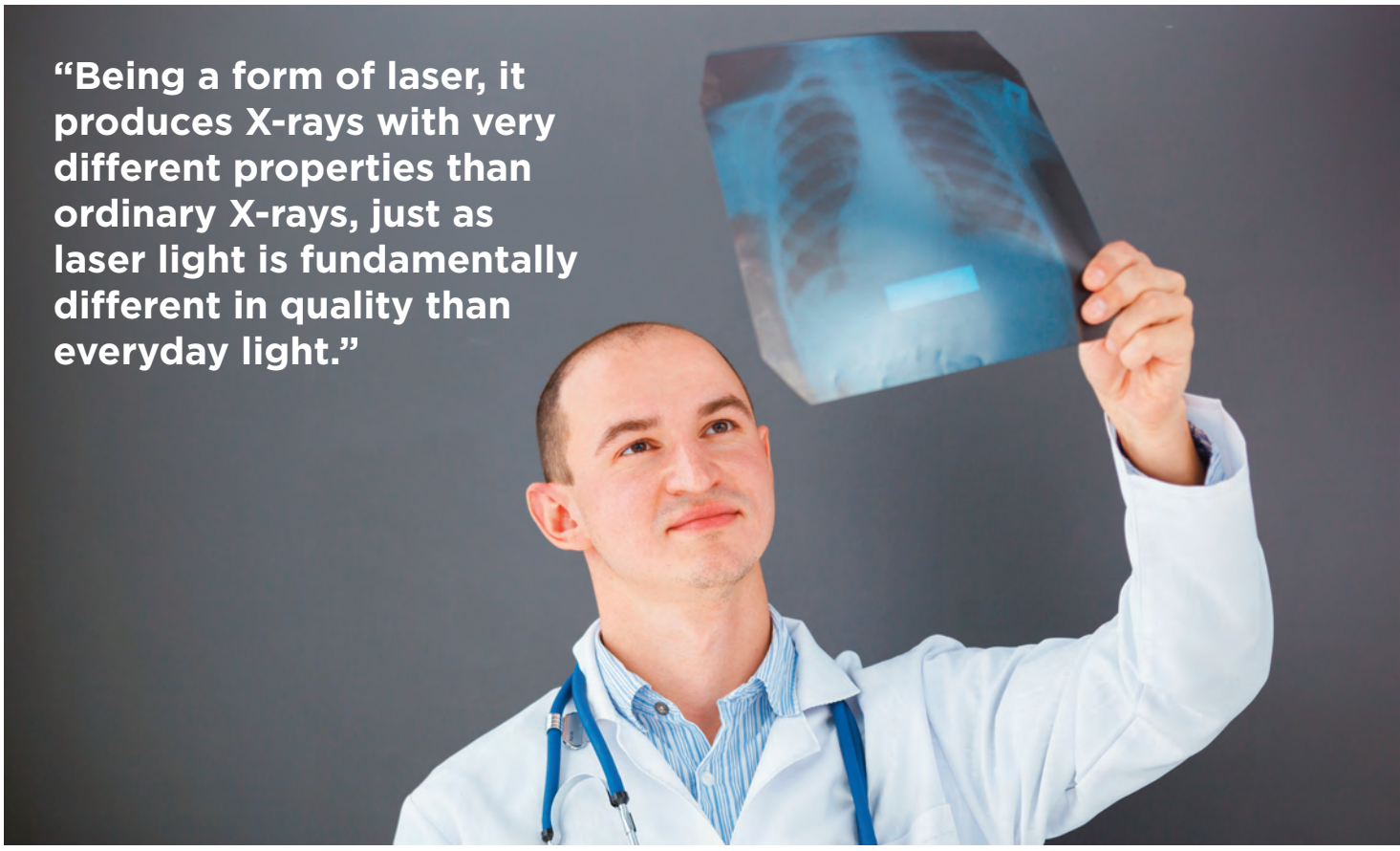
X-RAY LASER RESEARCH

Although the honor of the world's first X–ray free electron laser (XFEL) goes to the USA, there is due to come online a European XFEL in Hamburg in 2017, which will be superior in many ways to the one in the US (Stanford) as it will have a much higher repetition rate allowing many more diffraction patterns to be measured per experimental shift. This will allow novel applications that are not possible at the Stanford XFEL. It is perhaps fitting that the European XFEL is sited in Germany as a refection of the seriousness with which Germans take their scientific research, as the accompanying article makes clear.

Unfortunately, most of time-resolved crystallography to date happens in crystals [1]. A molecule is crystallized and the time resolved changes in the structure at very fast time scales can be studied by a time-resolved experiment using the difference Fourier method [2] which requires an identification of corresponding Bragg spots in an excited state and ground state crystal. The problem is that since all these reactions take place in crystals, steric hindrance may make the reactions of the molecules different from what takes place in real life where the molecules are swimming more or less freely in liquid water. Fortunately a method has been developed recently [3] that enables the visualization of chemical reactions of individual molecules even if they are not all aligned in the form of crystals.

Aiding this development has been the development of a brand new form of X-rays produced by a brand new instrument called an X-ray free electron laser (XFEL). Being a form of laser, it produces X-rays with very different properties than ordinary X-rays, just as laser light is fundamentally different in quality than everyday light. Indeed, an XFEL produces light that is so coherent that the brightness of X-rays from an XFEL is many orders of magnitude greater than X-rays from any previous source. Not only this, the X-rays come in pulses of the order of femtoseconds in duration. One of the great problems with the use of X-rays for the study of biological materials is that biomaterials are usually so fragile that radiation damage by the very probe X-rays is a problem. Indeed. with the use of ordinary X-rays, care must be taken to stay below a certain dose, so as to avoid the radiation damage problem. With XFEL radiation the hope is that the increased brightness will allow the use of radiation from essentially an unlimited number of pulses without the need to worry about the radiation damage problem (because it is unimportant if the scattering takes place over a shorter time than the time it takes for molecular disintegration). Therefore the X-rays see the undamaged crystal no matter how bright the X-rays. What happens to the molecule afterwards? It is of no relevance if we are only concerned with what the X-rays scatter off. This completely breaks the nexus between radiation damage and the limitations of X-ray methods for studying biomolecules.

Up to the development of the XFEL, almost all structure determination at the atomic scale was done on macroscopic crystals, where the relatively weak X-rays from conventional sources scatter off perhaps trillions of identical unit cells to reveal the structure of a single unit



cell. What is more, the relative fragility of biomolecules seemed to suggest a maximum X-ray dose for avoidance of radiation damage. All this changed with the development of the XFEL.

It was realized by Neutze et al. [4] that one could usefully exploit two features of the XFEL, namely its ultra brightness and the fact that x-rays are delivered in ultrashort pulses to perform atomic scale structure determination in completely novel environments. Firstly, the ultra brightness may allow structure determination to be performed on individual molecules without a need to crystallize them. This is of significance, as there are some important biomolecules, e.g. membrane proteins, that are very resistant to crystallization. In any case, a crystalline state is not one in which biomolecules perform their functions in nature. The ultra brightness of an XFEL will allow meaningful diffraction patterns to be measured from individual biomolecules in enviromnents closer to those in which they may be found in nature, e.g. in solution. The shortness of the x-ray pulse could allow one to outrun radiation damage which takes place over a time period longer than the x-ray pulse. Thus the x-ray diffraction patterns would be chractereristic of their native states before radiation damage. Thus one may use any degree of incident flux and be completely impervious to radiation damage!

Of course, the use of such completely different conditions for structure determination also requires the development of completely new theory. No longer can one use the theory originally developed by the Braggs, von Laue and other pioneers of X-ray crystallography which were designed for crystals [2]. In fact, X-ray diffraction patterns from individual

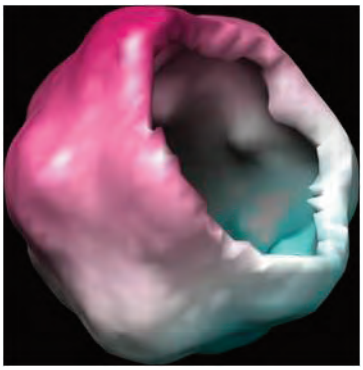


Figure 1 Structure of an icosahedral virus, satellite tobacco necrosis virus (STNV) recovered from simulated diffraction patterns by the method of angular correlations. The artificial cut allows us to show that the reconstruction of the PDB structure assumed in the calcuations, which contains only the capsid structure.

molecules look very different from those from crystals. The latter are dominated by the "Bragg spots" which arise to the crystalline periodicity. This is both a boon and a curse. The maximum intensities of the Bragg spots increase with the number of the number of "unit cells" in a crystal and make them much more visible even with much weaker X-rays. On the other hand the crystal periodicity prevents the sampling of these intensities at intervals smaller than the positions of the Bragg peaks. Although that scattering is weaker from an individual molecule the possibility of measuring such "oversampled" intensities is often crucial for structure determination. Here is the advantage of the 10 billion-fold increase in brightness of the incident X-rays allowed by the XFEL. However it should be borne in mind that a single molecule in perhaps a trillion times smaller than a typical crystal used in X-ray crystallography, so the measured intensities would inevitably be significantly smaller. This also necessitates a careful consideration of signal-to-noise ratios. Also, the absence of Bragg spots in scattering by individual molecules requires entirely new methods of taking account of the orientations of the individual molecules.

The simple fact is that droplets of solvent that are typically inserted into an XFEL beam are of the order of a micron in size while an individual protein is about an order of magnitude smaller. Consequently, the X-rays scatter off not one but a collection of particles. Unlike the trillions of molecules in a crystal which are all in identical orientations, molecules in a liquid will be in random orientations. It is possible to turn this to advantage by developing methods of analyzing the experimental data from ensembles of randomly oriented particles, and not just single particles as has been the focus of most theoretical methods up to now.

The key to the new method is an analysis not on the bare intensities from experiment, but on their angular correlations [5]. These correlations have been found to be characteristic of the structure of the particles despite their random orientations. We believe that the effect of the random water molecules can be taken care of by what is known as Babinet's principle, also used in the complementary method of small angle x-ray scattering (SAXS) [6], where due to the limited information from experiment has largely been confined to studying molecular shapes, rather than internal structure.

One of the earliest application of the method of angular correlation has been to work out the structures of the two commonest forms of regular virus [7] the icosahedral and helical. In both cases it is possible to develop methods that correctly recover the structures as shown here. Fig 1 shows the recovery of the capsid of the icosahedral satellite tobacco necrosis virus from angular correlations of the scattered intensities where the PDB file had been used

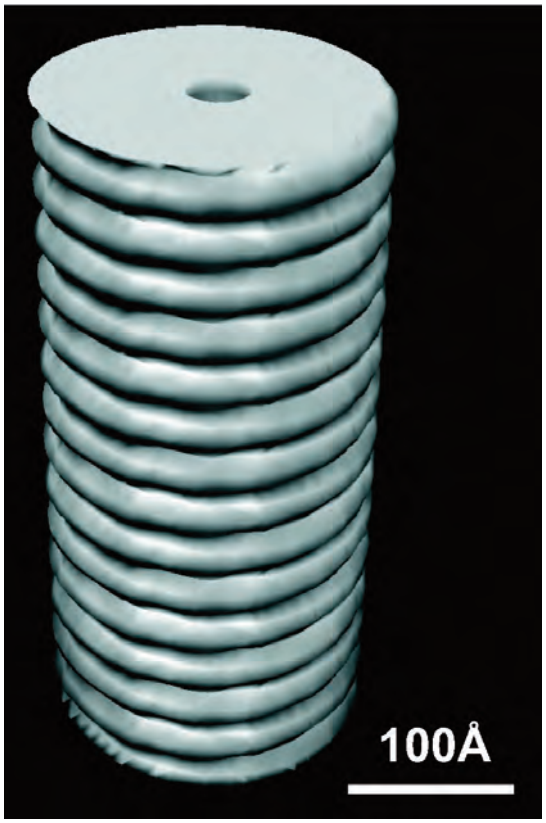


Figure 2 Structure of tobacco mosaic virus (TMV) recovered from simulated XFEL diffraction patterns by the method of angular correlations.

to simulate the diffaction patterns from the virus. The PDB file does not contain the internal genetic material. In addition be make a computational slice through the figure to show that the capid is hollow. Fig. 2 shows the recovery of the helical tobacco mosaic virus. A question that might justifiably be asked is what about irregular viruses. For example there is some evidence that the chlorella virus develops a spike on a unique vertex. We believe we can develop a perturbation approach that can show up the spike from experimental data, but that is a subject for the future.

Acknowlegements

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RESEARCH EXCELLENCE IN GERMANY

With the renegotiation of the Pact for Research and Innovation, Germany seeks to cement an international reputation for excellence in science, research and development, as Adjacent Government highlights...

Germany's science and innovation system is now well positioned – even by international standards. Never before has so much been invested in Germany in research and development (R&D). Between 2005 and 2013, nominal R&D spending in Germany rose by almost 70%.

In a speech Federal Minister for Education and Research, Johanna Wanka detailed further how reliability for funding in research is integral to achieve excellence:

“How the money is used is equally important, and even more important for science is reliability. Through the Pact for Research and Innovation, we guarantee reliability for extramural research institutions, which in itself is very valuable.

During the coalition negotiations, we were in a difficult situation. A number of states said that they were not prepared and did not feel able to continue the pact because of their financial situation. Some also referred to their own colleges, which would also be strengthened. At this point, it was only right and very important that the federal government has committed to carrying on the Pact for Research and Innovation alone.

In Germany we have achieved a very high standard throughout the higher education system. The Excellence Initiative has brought movement into the system and moved us to measure outcomes.

“Important decisions lie ahead of us in the education and research policies we will make, in consensus with our partners in the scientific landscape.”

You must understand that I do not want to prejudge the results of Imboden Commission which is currently evaluating the Excellence Initiative. I would like to decide in the first quarter of next year what will happen next. It is existential for Germany to promote excellence. I am therefore grateful for all suggestions that are made.

With academic freedom and academic responsibility, the scientific elite is growing. The career paths within the science system are in need of reform. Since 2005 a lot of money has come into the system, usually temporarily. This was associated with a huge increase in short-term contracts. The balance between permanent professorial jobs and temporary jobs is out of kilter. Do not misunderstand me: A scientific system needs both. Too many

permanent jobs makes the system inefficient, at the expense of the next generation. But it is no longer right that we should have as many temporary positions as we do at the moment.

With the BAföG [Federal Training Assistance Act] changes we have already taken the first step. Thus, states now have a reliable income for permanent positions. The Science Council proposed a very satisfactory model of career goals and paths at universities last year.

Many large research infrastructures are not purely national, but occur in cooperation with numerous partners in Europe and worldwide. That means that any decision on German involvement in these projects needs a thoughtful and well-reasoned basis, guaranteeing reliability to our international partners. Important decisions lie ahead of us in the education and research policies we will make, in consensus with our partners in the scientific landscape."

"Never before has so much been invested in Germany in research and development (R&D). Between 2005 and 2013, nominal R&D spending in Germany rose by almost 70%."

The above is an abridged translation of the speech delivered at the annual meeting of the Max Planck Society on 18 June 2015. For further information please visit https://www.bmbf.de/

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