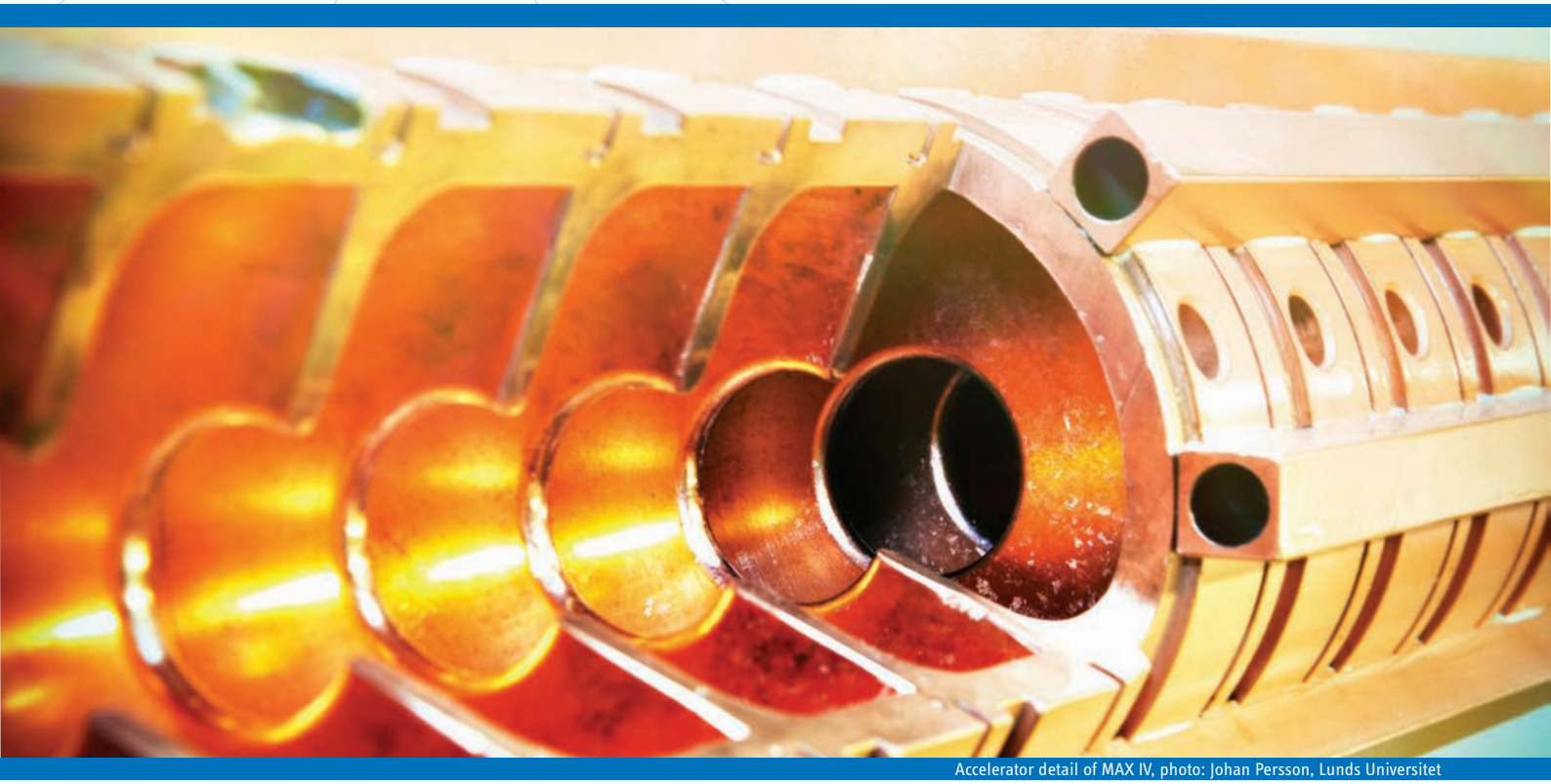
Röntgen Ångström Cluster

NEWSLETTER 09 | JUNE 2014



Accelerator detail of MAX IV, photo: Johan Persson, Lunds Universitet

FIRST ELECTRON PULSES IN MAX IV

WORK ON THE NEW FACILITY WELL ON SCHEDULE

The Max IV facility in Lund, Sweden, has produced its first electron pulses in May this year. “It was great to see that everything worked as it should”, said Sara Thorin, project manager for the linear accelerator. Thorin and her team, consisting of numerous scientists and engineers, concluded this part of their mission well on schedule after devoting the past six months to it.

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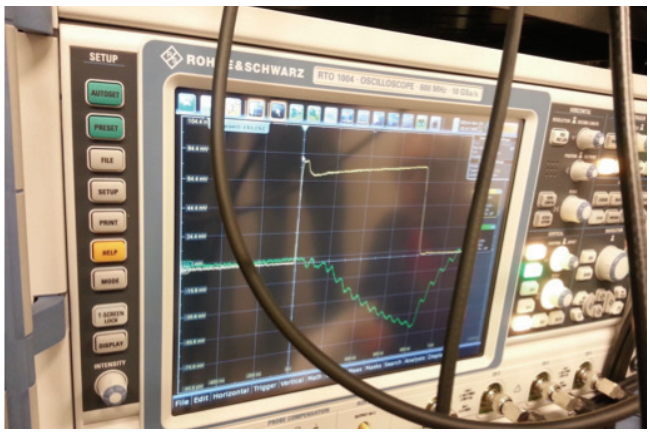


First electrons running at MAX IV: The linear accelerator group has turned up the temperature of the cathode producing electrons in the gun. Everything worked just as it should, and out came the ten longed-for electron pulses per second. Photo: MAX IV

EDITORIAL

“None of us gets bored”, Christoph Quitmann told us over the phone, laughing. We managed to get hold of the very busy director of MAX IV in the waiting lounge of Charles de Gaulle Airport in Paris. From there, he gave us an update on the developments at the exciting new facility-in-the-making in Lund. Quitmann’s comment can safely be applied to most Swedish and German scientists working in the context of the Röntgen-Ångström-Cluster as you will see in this newsletter. Winfried Hinrichs and Uwe Bornscheuer of the University of Greifswald have made use of the excellent research opportunities for molecular structural biology in Berlin. See on page 2 for their new findings.

We’re also pleased to start a new series of snapshots of projects funded by the Röntgen-Angström-Cluster. Please get in touch if you’re involved in one. We’d love to hear from you. Viel Spaß! Ha det så kul! (Enjoy!)

Make sure to visit www.rontgen-angstrom.eu regularly. It’s an easy way to keep yourself informed about news, deadlines, forms, workshops, people, and projects!

The Editors

EXPANDING THE TOOLBOX FOR THE INDUSTRIAL APPLICATION OF TRANSAMINASES

Gap filled: structural data of (R)-selective amine transaminases is available


Winfried Hinrichs

State-of-the-art chemistry works with detailed information concerning the 3-dimensional arrangement and interaction of atoms. This became possible since about a century ago when X-ray diffraction on single crystals was demonstrated and crystallography provided the atomic structures of table salt and other chemical compounds with increasing complexity. Presently also biological objects like viruses, photosystems and ribosomes are the objects of interest. The enormous impact of crystallography, of course not only single crystal diffraction, within natural sciences from physics, chemistry to biology and applications in biotechnology, pharmacy and molecular medicine is actually dignified by the UNESCO highlighting the year 2014 as "International Year of Crystallography".

Many organic molecules occur in an original and its mirror image. They are related to each other like the left hand to the right. Most of the biological molecules possess the so-called chirality or handedness and organisms strictly distinguish between these two, the (R)- or (S)-stereo-isomers. Thus, correct handedness of molecules play an important role in pharmaceutical research and applications. High purity of these chemical compounds, also with respect to stereo-isomers, is mandatory for clinical applications.

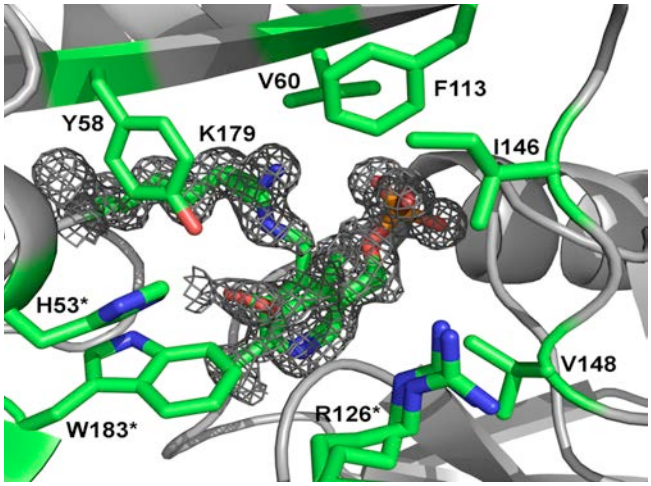
In biotechnology, enzymes are in use as common catalytic tools to produce high-value products for pharmaceuticals. Nowadays, specifically designed enzymes catalyze reactions in certain production processes. These efforts need "rational protein design": tailoring of specific enzymes which catalyze the production of biological or non-biological compounds for direct applications in biotechnology and pharmaceutical industry. Rational protein design is based on detailed knowledge of the 3-dimensional atomic structure of the enzyme of interest, which is essential to understand the mechanism of the catalyzed chemical reaction. The method of choice to get this information is X-ray crystallography.

A nice example are transaminases, which convert prochiral precursors only to one of the possible stereo-isomers of amines. These compounds are applied as synthons or ingredients in agro-chemistry, medicine, pharmacy and basic materials industry. Crystal structures of (S)-selective amine transaminases have been described in detail, but structural data of (R)-selective amine transaminases were not available. The (R)- and (S)-selective amine transaminases share no significant amino-acid sequence identity, preventing successful structure modeling.

This gap for industrial applications has been filled by a joint project of the groups of Molecular Structural Biology (Winfried Hinrichs) and Enzymology (Uwe Bornscheuer) both at the Biochemistry Institute of the University of Greifswald. The (R)-selective amine transaminase from Aspergillus fumigatus was expressed recombinantly, purified and crystallized. X-ray diffraction data collection was carried out at 100 K on beamline 14.1 of the Helmholtz-Zentrum Berlin at the BESSY II synchrotron radiation source, Berlin, Germany. Here, we have one of countless examples demonstrating excellent research opportunities of large-scale facilities for molecular structural biology.
The structure was solved by S-SAD phasing to 1.84 Å resolution and final diffraction data led to a refined model at 1.27 Å resolution of the fungal (R)-selective amine transaminase. This structure determination is part of the Ph. D. thesis of Maren Thomsen (M. Thomsen et al., Acta Cryst. (2014). D70, 1086–1093).

The active site of the enzyme shows how substrate recognition occurs in (R)-selective amine transaminases. Now, the enzyme structure will be used to modify stereo-specific substrate recognition by site-directed mutagenesis. This expands the toolbox for the industrial application of transaminases significantly and enables selective production of each enantiomer with designed (R)- or (S)-selective enzymes.

We thank Winfried Hinrichs for his Scientist's contribution.



Detail of the active site of the (R)-selective amine transaminase: The catalytic active centre is surrounded by several amino acid side chains (one-letter code and numbering). The typical pyridoxal-5'-phosphate cofactor bound to the lysine side-chain (K179) is highlighted with its corresponding electron density map (cage network). The colour code of the atoms in the stick model is as following: green – carbon, red – oxygen, and blue – nitrogen atoms. © Winfried Hinrichs

PROJECTS

Starting with this edition, we aim to introduce some of the projects supported by the Röntgen-Ångström-Cluster in each newsletter to come. If you are working on a project and would like to share your progress, please contact us at editor@rontgen-angstrom.eu.

DIMETAL-CARBOXYLATE CATALYSIS IN ENZYMES AND BIOMIMETIC MATERIALS STUDIED BY NOVEL X-RAY CRYSTALLOGRAPHY AND SPECTROSCOPY TECHNIQUES


Michael Haumann

Project Partners: PD Dr. Michael Haumann (Coordinator), Freie Universität Berlin; Prof. Volker Schünemann, Technische Universität Kaiserslautern; Prof. Martin Högbom, Stockholm University; Dr. Sascha Ott, Uppsala University

Proteins containing transition metal cofactors are of outstanding biological relevance. Their biological reactions are of prime interest also for technical applications in renewable energy, chemical industry, and medicine. A cofactor containing two metal ions coordinated by carboxylate groups in a highly conserved amino acid fold (dimetal-carboxylate cofactor, DMC) defines a large and diverse superfamily of proteins. DMC enzymes perform some of the chemically most demanding reactions in nature, for example the selective oxidation of methane to methanol, listed among the top-ten challenges for catalysis. Prominent members of the group are ribonucleotide reductases involved in DNA synthesis and monooxygenases with widespread substrate specificity. Mimicking the green chemistry of these systems is an outstanding goal for bioinorganic and organometallic chemists. Our understanding of the molecular, electronic, and vibrational properties of DMCs at present is insufficient to explain their various functionalities.

The archetypical DMC consists of two iron atoms (FeFe), but very recently, enzymes have been discovered, which instead bind MnFe or even MnMn cofactors. Their primary function is similar, namely activation of O2 at the entry point of electron transfer, radical formation, and substrate oxidation reactions, involving crucial reaction intermediates with high-valent metal ions. The reasons for the different metallations of the binding sites, as well as the structural and electronic differences between them are widely unclear. In the trans-disciplinary project, we aim at gaining deeper insight in the reaction mechanisms of the biological systems that potentially will lead to new biomimetic catalysts by linking structural biology and functional organometallic materials with development and application of novel synchrotron-based X-ray crystallography and spectroscopy techniques.

Experiments at the new facilities PETRA III (DESY, Hamburg) and MAX IV (Lund, Sweden), as well as at free electron laser sources are combined to obtain complementary information on reaction intermediates. X-ray absorption and emission spectroscopy (XAE) provides electronic configurations and fine structures. Nuclear resonance scattering (NRS) on isotopically labeled systems discloses vibrational dynamics and protonation events at the cofactors. Free electron laser femtosecond protein crystallography (FFC) will be employed to obtain molecular structures of high-valent states of wildtype and mutated proteins. Synthetic chemistry is used to implement the crucial features of the biological systems into tailored molecules. We will improve the instrumentation at two beamlines (P01 and P64) of PETRA III for a broader user community and gain experience on the application of free electron laser experiments to high-valent metal centers in proteins to be used at the upcoming sources (European XFEL, MAX IV). Integration of the spectroscopic and structural data with quantum chemical calculations will yield atomic-level models and reaction mechanisms for the DMC systems. This unprecedented information will significantly improve our fundamental understanding of the metal specificity, redox tuning, and functional diversity of this class of transition metal catalysts.

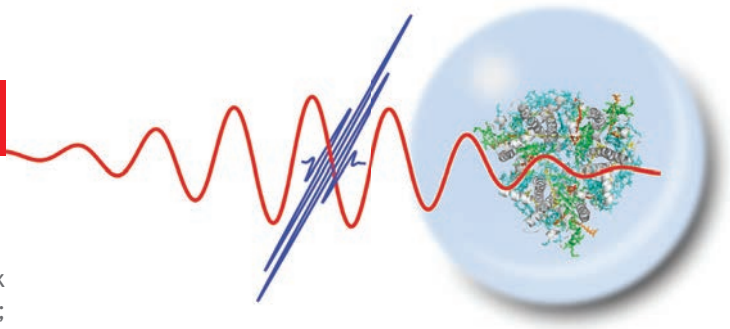
COFACTOR
A cofactor is a non-protein chemical compound which is required for the biological activity of a protein. These proteins are commonly enzymes, and a cofactor can be considered as helper molecules that assists in biochemical transformations.

REDOX
"Redox" comes from REDuction and OXidation and refers to atoms' change of oxidation state in chemical reactions. In electron transfer, a reduction is a decrease in oxidation state by a molecule, ion, or atom which causes a gain of electrons, whereas oxidation means the increase in oxidation state which makes a loss of electrons.

NEWS

A Swedish-German research team has successfully tested a new method for the production of ultra-strong cellulose fibres at DESY's research light source PETRA III. The novel procedure spins extremely tough filaments from tiny cellulose fibrils by aligning them all in parallel during the production process. The new method is reported in the scientific journal Nature Communications and you can read more about it at http://www.rontgen-angstrom.eu/go/cellulose

PROJECTS



Symbolic view of a water nano particle doped with a bio molecule. © AG Krikunova

CONTROLLING SAMPLE INTEGRITY IN FEL EXPERIMENTS:
Exposure, heating, and plasma dynamics in the time domain
Project Coordinator: Prof. Maria Krikunova, Institut für Optik und Atomare Physik, Technische Universität Berlin, Germany; Dr. Jakob Andreasson, Department of Cell and Molecular Biology, Uppsala Universitet, Sweden

Maria Krikunova

Jakob Andreasson

Free-Electron Lasers offer the possibility of single-shot imaging of free particles in the gas phase. In its fully developed form, this technology will bypass the current necessity to arrange sample particles in large high-quality crystals. Instead, scientists will be able to study structure and dynamics in complex biomolecules directly. Due to the high power density of the FEL radiation needed for high resolution Coherent Diffractive Imaging (CDI), the sample will be turned into a highly excited plasma after exposure. Thus, the electron dynamics eventually followed by the ion displacement in the sample during the pulse directly affect the formation of the diffraction pattern and put an ultimate limit to the obtainable resolution. Furthermore, the sensitivity of structure and function of biological samples to changes in their environment requires new concepts for sample delivery and precise control under high vacuum conditions.
The four-year research program within the Röntgen-Ångström-Cluster will refine the sample-delivery techniques that allow for handling of inorganic, organic and biological nanostructures. It will also develop the time-resolved methods to track the dynamics of nanometer- to micrometer-sized plasmas on ultra-short time scales. Within the collaboration we will focus on the development of:
(i) a cluster source for production of water nano-particles which could be doped with a variety of substances, including proteins and other macromolecules;
(ii) a sample delivery system capable of trapping and manipulating individual droplets in an interaction region where they can be held captive during the exposure;
(iii) key experimental and theoretical components for the study of the dynamics of nano-plasma formation using terahertz or near-infrared radiation combined with X-ray FEL pulses with high temporal and spatial resolution;
(iv) computer modeling for studies of matter in extreme conditions and methods for handling the vast quantities of data generated by e.g. the European XFEL.
The research project brings together the complementary experimental and theoretical expertise of the research groups from Uppsala and Berlin in the fields of CDI, handling of biological samples and computer simulations to address the development of new time-resolved techniques for studies of ionization dynamics of small quantum systems. The results from this collaboration will contribute to the understanding of the physical processes that govern the response of matter under extreme irradiation conditions. Furthermore, it will enhance the exploration of the full potential of Swedish-German research infrastructures: MAX IV, FLASH as well as the European XFEL.

INTERVIEW

WE ARE THE COOLEST SHOW IN TOWN

Christoph Quitmann about the MAX IV facility in Lund

MAX IV has just started to commission the linac. Are you pleased with your team's work?
Yes, very pleased. The team did a fantastic job. This is the first real step from design to realization. There were more than ten thousand technical issues to be solved, and the team solved them. I'm proud of it.

You arrived in Lund about two years ago from Switzerland. Can you recall your first impressions of your new work place?
Yes, I have always found this to be a cool project. Seeing the reality and the people around it was a real revelation. This is the coolest show in town, a landmark. My first impression was real excitement.

How does Sweden compare to Switzerland in terms of work, concerning your particular field?
It's the same, but different. Many things are similar. The technical challenges for example are the same. But the resources in Lund are much more limited. We operate in a different mode. We have a team of 160 people – that is a very small number. Having said that, I'm impressed with the spirit of the people to pull off such a project with limited resources. Another difference is that the facility in Switzerland was located in the woods. In Lund, we're very close to the University. This has an impact on the atmosphere, it's more vibrant.

Tell us about your achievements in Lund so far.
The Linac is operational. The outside of the building is completed. We're working on installing the magnet rings. Most importantly: we managed to build trust and confidence in the Swedish and the international science community. We're no longer a project on paper. People take us seriously. Brasil and Japan, for example, watch us closely and copy some of our methods. We're proud of that but the attention also builds up the pressure.

What are your next challenges?
They are clear. We need to complete the installation and the building. We also have a facility which is currently operating and handling about 1000 users per year until the end of 2015. None of us gets bored.

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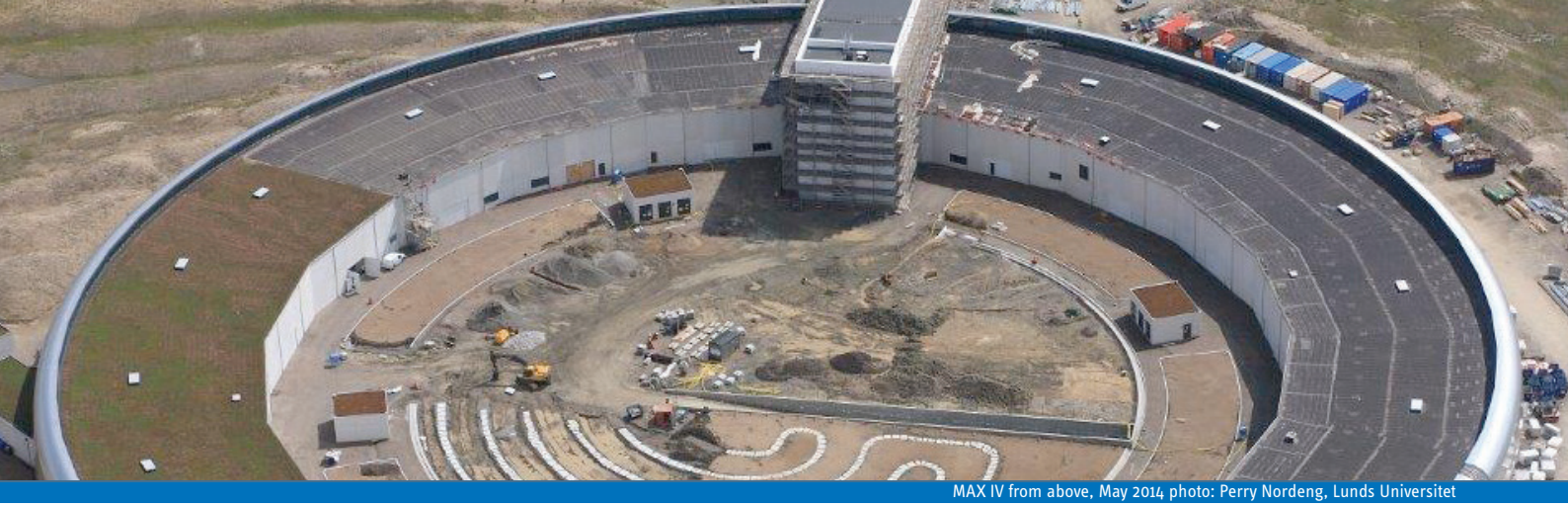

MAX IV from above, May 2014 photo: Perry Nordeng, Lunds Universitet

INTERVIEW

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The new facility is scheduled to open in June 2016. Will it open on time?
Yes, it will. There is no indication of a delay. We are confident to open on Midsummer's Day 2016, Tuesday, 21st of June at 1 o'clock. This is an important time for Sweden. And we realized it was important to have a target date in mind. So we decided to use the brightest moment in the year to open the brightest X-ray source in the world.

How will MAX IV change the scientific landscape?
Already now, MAX IV has had an impact. We're making possible what previously wasn't. We will provide more information with less radiation damage. But it can't be done by us alone. We need the best users from around the world to bring their best projects to Lund.

A personal question: What do you value most about life in Sweden? Is there anything you can't adjust to? Do you find the time to learn Swedish?
I like the people. They are very friendly. I love the Swedish light in the summer. But as a keen mountain biker and climber I miss the mountains. I try to learn Swedish but the Swedish make it very hard for me. Everybody speaks perfect English, so there's little driving force. However, I have recently discovered Astrid Lindgren movies I used to watch as a child in Swedish with English subtitles. That might help me learn.

FIRST ELECTRON PULSES IN MAX IV

(continued from page 1)

The next challenge will be to tune all magnets, radio frequency components, diagnostic and other systems so that electron pulses can be shaped and transported as needed when sent onwards to produce light. Eventually, the electrons will be sent into the storage rings or the short pulse facility.
The large storage ring is expected to be installed in July 2015 and the small storage ring in January 2016. Commissioning of the entire facility is scheduled to commence in the second half of 2015 and the inauguration of MAX IV will take place on June 21st in 2016.

ANNOUNCEMENTS

SECOND RACIRI SUMMER SCHOOL

The RACIRI summer school is a joint German-Swedish-Russian initiative to promote the next generation of scientists with a strong connection to the large-scale research infrastructures (synchrotrons, lasers, neutrons) in the Baltic area. The first RACIRI Summer School on advanced materials design was successfully held in Peterhof, Russia, in 2013.
This year's RACIRI Summer School will be organized from 24 to 31 August 2014 on "Imaging with X-rays and Neutrons in Life and Materials Sciences" in the greater Stockholm area, Sweden. To get more info, please visit the section "announcements" on www.rontgen-angstrom.eu.
The RACIRI summer school offers scholarships for students/scholars from universities/research institutions from Germany, Sweden and Russia.

FIRST NORDIC X-RAY SCIENCE DAYS

Opportunity to get together with the large user community
MAX IV Laboratory announces the first Nordic X-Ray Science Days to take place from 27th of September until the 1st of October this year (2014). Traditionally, MAX LAB IV used to organize the Annual MAX IV Laboratory User Meeting (UM14) in cooperation with The Association for Synchrotron Radiation Users at MAX-lab (FASM). This year, the event has been extended to include a much larger user community. The aim of the Nordic X-Ray Science Days is to engage all Nordic X-ray users and to build more bridges between the various institutions committed to X-Ray sciences. The event takes place in Lund, Sweden, Downtown University Campus.

Please visit www.rontgen-angstrom.eu to get more information.

IMPRINT

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