In the news

Luciferase throws light on heart disease

For the first time ever, researchers have directly measured energy levels inside living heart-muscle cells (cardiomyocytes) by using an adenoviral system to express photoproteins luciferase and aequorin, targeted to the mitochondria or cytosol of adult cardiomyocytes, in order to investigate the interrelationship between adenosine triphosphate (ATP) and Ca^{2+} in these compartments and gain insight into heart disease [1].

''Being able to see exactly what's going on in heart cells will be of great benefit to understanding heart disease,'' said Elinor Griffiths of Bristol Heart Institute, UK, who worked with colleagues on the research that could lead to improved recovery of the heart when it is re-started after a heart attack or cardiac surgery.

Mitochondria convert energy from food into ATP, and, under normal conditions, they can make ATP extremely rapidly when the heart is stressed, such as during exercise or in the ''fight-or-flight'' mode. However, if the cardiomyocytes are made to beat suddenly from rest, as happens when the heart is re-started after cardiac surgery or a heart attack, Griffiths and colleagues found that there is a lag phase where the supply of ATP drops before mitochondrial production starts again, potentially preventing the heart from beating properly. When mitochondria do not produce enough ATP to keep the cardiomyocytes alive, they die and collapse (Fig. 1).

''The breakthrough presented by this technique could be of benefit in heart diseases where mitochondria cannot make enough ATP,'' said Griffiths. ''When that happens, the heart does not have enough energy to perform its function of pumping blood efficiently and that can result in a heart attack.''

Exactly how mitochondria tailor the supply of ATP to demand is not fully known. However, being able to directly measure ATP levels inside mitochondria of living cardiomyocytes in real time will help to advance understanding.

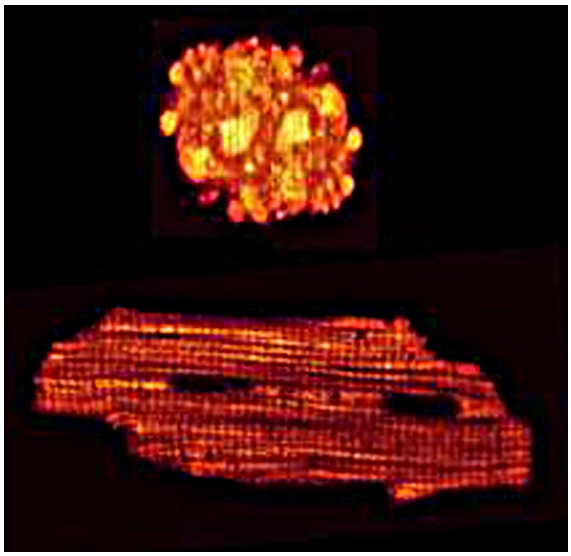

Figure 1. Live (left) and dead heart cells. When mitochondria do not produce enough adenosine triphosphate (ATP) to keep the cells alive, they die and collapse.

Reference

[1] C.J. Bell, N.A. Bright, G.A. Rutter, E.J. Griffiths, J. Biol. Chem. 281 (2006) 28058.

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Enzymic digestion confirms peanut allergens

The US Food and Drug Administration laboratory in Maryland has found a method for unambiguously confirming the presence of peanut allergens in dark chocolate by combining enzymic digestion with high-performance liquid chromatography-electrospray ionization tandem mass spectrometry (HPLC-ESI-MS^2) [1]. Dark chocolate is potentially dangerous for anyone allergic to peanuts, but is an awkward matrix to deal with.

Kevin J. Shefcheck and colleagues used the mass of key peptides and their amino composition as definitive signatures for the protein allergens from which they originated. The

advantage of the enzymic digestion is that the measurement regime is shifted from large, complex molecules and their associated interactions, to smaller molecules with fewer matrix interactions that need only simpler chromatographic techniques.

They identified and measured peanut protein in dark chocolate at concentrations as low as 2 ppm. Analysis of the selected markers provided two tiers of identification and confirmation for peanut protein in chocolate:

- the retention time and mass of Ara h 1 peptide markers indicated the presence of the allergen; and,
- mass selection and fragmentation of the peptide generated structurally indicative ions, confirming the identity of the peptide and the protein from which it was derived.

Even lower detection levels could be achieved by digesting the peanut protein during extraction from the chocolate, rather than extracting the protein first and then digesting it.

Shefcheck and colleagues consider that, by developing suitable internal standards, it will be possible to use this as a quantitative method that could be adapted to detect and quantify other allergenic proteins in other food matrices.

Reference

[1] K.J. Shefcheck, J. Agric. Food Chem. (2006).

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Protein protects against Parkinson's disease

Fluorescence lifetime imaging (FLIM) has revealed how the chaperone protein Hsp70 acts within cells to block the formation of molecules that cause neurodegenerative diseases, such as Parkinson's disease (PD) and dementia [1].

Oligomerization and aggregation of α-synuclein molecules are believed to play a major role in neuronal dysfunction and loss in PD and dementia with Lewy bodies. Until now, α-synuclein oligomerization and aggregation have been detected only indirectly in cells using detergent extraction methods.

However, Jochen Klucken and colleagues at the Department of Neurology, University of Regensburg, Regensburg, Germany, and Alzheimer's Disease Research Unit, Massachusetts General Hospital, Charlestown, Massachusetts, USA, used FLIM to show for the first time intracellular α-synuclein oligomerization. They detected two forms of α-synuclein homomeric interactions: an anti-parallel amino terminus-carboxyl terminus interaction between α-synuclein molecules; and, a close amino terminus-carboxy terminus interaction within single α-synuclein molecules.

In addition, they demonstrated that coexpression of the chaperone protein, Hsp70, which can block α-synuclein toxicity in several systems, caused α-synuclein to adopt a different, open conformation. However, they also showed that Hsp70 did not alter α-synuclein–α-synuclein interactions.

Klucken and co-workers therefore concluded that the neuroprotective effect of Hsp70 against neurodegenerative diseases, such as PD and Alzheimer's, could be explained by the chaperone activity of Hsp70 on α-synuclein molecules, rather than alteration of α-synuclein–α-synuclein interactions.

Reference

[1] J. Klucken, T.F. Outeiro, P. Nguyen, P.J. McLean, B.T. Hyman, FASEB J. 20 (2006) 2050.

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ESRI will view organs in 3-D

Scientists, engineers and mathematicians at the University of Bath, UK, last month started a fundamental revision of electron spin resonance imaging (ESRI), with a view to dramatically improving understanding of serious illnesses, such as heart disease, stroke, diabetes, septic shock and cancer.

They hope that ESRI will eventually make a three-dimensional (3-D) ''snapshot'' image of the chemical state of an organ such as the heart. At present, instruments do not have the sensitivity or the speed to do this. But, using the latest research into measurement techniques and data analysis could improve sensitivity by 100 times or more, so that some images could be recorded 10,000 times faster or with 10,000 times more spatial information.

ESRI instruments work in a similar way to magnetic resonance imaging (MRI) body scanners that are already widely used in hospitals. However, MRI uses the magnetic properties of protons in water to generate an image, whereas ESRI uses the magnetic properties of electrons. This fundamental difference makes ESRI more suited to imaging chemical processes. However, ESRI is technically much more difficult, so its use has been restricted to the research laboratory.

The four-year, €1.25m ($1.6m) project is funded by the Biotechnology and Biological Sciences and the Engineering and Physical Sciences Research Councils of the UK.

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Single-molecule sensor will detect disease

Scientists at the University of Southampton, UK, aim to detect disease using a device to identify molecules more sensitively than ever before.

Professor Hywel Morgan at the School of Electronics & Computer Science and Peter Roach at the School of Chemistry and their team have received a European grant of €450k ($570k) to create a system that can detect single molecules in biological solutions. They aim to create ''senses'' by binding together variants of biological molecules to mimic the human nose. With this approach, they believe that the sensitivity of their device can be 1000 times better than currently available electronic noses.

The receptors, which will be housed within an artificial membrane, remain in a closed, steady state until approached by smell molecules, when they will open and transmit an electrical signal that will indicate the nature of the odor.

''Many medical diseases involve odor,'' explained Professor Morgan. ''A device such as ours could measure different hormones, diagnose diseases and even sniff for traces of explosives. Most odors are still mapped by humans. If we can find a way to replace this function with technology, we could use odor detection in many new areas.''

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IC launches NMR facility

Sir Richard Sykes, Rector of Imperial College (IC) London, has opened a world-class, cross-faculty centre for nuclear magnetic resonance (NMR) spectrometry and the complementary Waters Laboratory of Molecular Spectroscopy.

Funded by the UK's Science Research Investment Fund, the €7.4m ($9.4m) NMR facility features an extremely powerful shielded 800 MHz magnet from Bruker BioSpin for studying the composition, structure and interactions of complex molecules at high resolution. The Waters Laboratory of Molecular Spectroscopy includes $1m of instrumentation funded by the Waters Corporation – the Acquity ultra performance liquid chromatography (UPLC) system (Fig. 2), and the Q-Tof Premier and GCT Premier mass spectrometers.

''NMR holds the key to unprecedented insight into fundamental processes in medicine, biology, chemistry and materials,'' explained Professor Steve Matthews, Division of Molecular Biosciences, IC. ''Our new NMR spectrometer here at Imperial is extremely powerful and will enable researchers from across the College to understand molecular structures and dynamics in greater detail than ever before.''

Jeremy Nicholson, head of the Department of Biomolecular Medicine, IC, thanked Waters Corporation for support.

''I'm delighted to welcome Arthur Caputo and his colleagues from Waters Corporation who have traveled from the States to attend the official opening of the Waters Laboratory of Molecular Spectroscopy,'' he said. ''Their investment in the new mass spectrometry laboratory – together with our new NMR facility – means that the College has a uniquely powerful new facility for molecular structure elucidation, which will enable researchers here in the future to develop new disease diagnostics based on small-molecule biomarkers and to understand molecular mechanisms of disease.''

''For the last four years, Waters has enjoyed a strong relationship with Imperial College London,'' said Art Caputo, President of the Waters Division. ''During that time, we

Figure 2. Sir Richard Sykes, Imperial College London (left) and Art Caputo, President of the Waters Division, look over the Acquity ultra performance liquid chromatography (UPLC) system.

have worked on honing our expertise in the area of chemometric data analysis for metabonomics and biomedical applications and worked on strategies for creating more useful liquid chromatography and mass spectrometry tools for systems biology and structural characterization of biomolecules. We are delighted to be able to assist in continuing the important biological research with them and look forward to building on this relationship in the future.''

SPE-HPLC-ESI-MS^2 pinpoints priority pharmaceuticals

Solid-phase extraction (SPE) isolation has been successfully combined with high-performance liquid chromatography-electrospray ionization tandem mass spectrometry (HPLC-ESI-MS^2) to determine fluoxetine (Prozac) and 10 other pharmaceuticals listed on the Oslo and Paris Commission for the Protection of the Marine Environment of the North East Atlantic (OSPAR) hazardous substances website [1].

Paul Roberts and Philippe Bersuder of the Centre for Environment, Fisheries and Aquaculture Science, UK, developed the simple extraction and analytical procedure because these sorts of pharmaceutical compounds, including antipsychotics and tranquilizers, are of possible environmental concern in surface-water and sewage-effluent samples.

They first tested a number of SPE sorbents, from which they selected Phenomenex's Strata-X for further development. They obtained recoveries of over 60% for most of the compounds and relative standard deviations of 4–13%, although recoveries of chloroquine and closantel were below 50% and the method provided only semi-quantitative information regarding their occurrence.

Roberts and Bersuder separated the analytes using Phenomenex's C_{18} Luna analytical column in ThermoFinnigan's Surveyor HPLC system, and then obtained the mass spectra using ThermoFinnigan's LCQ Advantage ion trap mass spectrometer equipped with an ESI probe operated in both positive and negative ionization modes. The limits of detection (LODs) for all compounds were in the range 1–20 ng/l, so the method was suitable for low-level environmental analysis.

They then used the method to measure the concentrations of the 11 compounds in six selected samples – Crossness surface water (2), Rodbourne effluent (2), Rodbourne downstream and Thames Barrier. They detected chlorpromazine, fluoxetine and miconazole at concentrations in the range 7–34 ng/l, with fluoxetine (<20–34 ng/l) showing the highest concentrations in all six selected samples, but highest of all (34 ng/l) in one of the sewage-effluent samples.

The method displayed good reproducibility, sensitivity and selectivity to the variety of pharmaceutical structures.

''The low limits of detection and recoveries in combination with the derived data show that the methods are suitable for environmental monitoring and in the future will be utilized to establish the concentration of selected compounds in the marine environment,'' concluded Roberts and Bersuder.

Reference

[1] P.H. Roberts, P. Bersuder, J. Chromatogr., A (2006) (doi: 10.1016/j.chroma.2006.08.093).

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Swedish partners support analytical center

Researchers at Uppsala University in Sweden have the opportunity to develop new methods of analysis for neurodegenerative diseases, in a collaborative effort with Akademiska University Hospital, GE Healthcare, AstraZeneca, Olink Bioscience, Affibody and Gyros.

''A long-term investment in interdisciplinary basic research in this field provides Uppsala with a unique opportunity that will hopefully contribute both to medical advances and economic growth in the region,'' said Fredrik Nikolajeff, associate professor at the Ångström Laboratory and coordinator for this Berzelii Center, the first of four that will be funded by VINNOVA (the Swedish Governmental Agency for Innovative Systems) and the Swedish Research Council in support of excellent research environments with strong ties to the business community.

Uppsala Berzelii Center will develop new analytical methods for protein-based diagnostics and screening of complex neuro-related disorders. It will combine research in biology, chemistry, pharmacy, medicine, materials science, and nanotechnology.

One key goal is for the methods to be able to provide enhanced knowledge of what biomarkers can be used for early diagnosis, a field where knowledge is limited today.

''This is a major problem for both health care and the drug companies,'' said Nikolajeff. ''Unfortunately, today complex diseases, such as Alzheimer's and Parkinson's, are discovered only in their late phases. If we knew what markers signal a risk of developing a certain neurodegenerative disorder, we would be able to use those markers for early diagnosis and perhaps large-scale screening, as is done for breast cancer in women (mammography).

''With early diagnosis, it is moreover easier to administer effective therapy based on each individual response,'' he said.

The Center will be financed for 10 years with a maximum of SEK100m from the Swedish Research Council and VINNOVA, with at least SEK70m from the University, the business community, and the public sector.

MHS-SPME-GC-ECD-ICP-MS spots suspect solvents

Multiple headspace solid-phase microextraction (MHS-SPME) followed by on-line coupling of gas chromatography with electron capture detection (GC-ECD) and with inductively coupled plasma mass spectrometry (ICP-MS) can be optimized to determine halogenated solvent residues in edible oils [1].

The adverse influence of these carcinogenic residues on the quality of edible oils as well as on human health led the European Union to impose restrictive regulations that limit them individually to 0.1/mg/kg and their total to 0.2mg/kg. As a result, new analytical methods for sensitive, precise determination were needed, such as this MHS-SPME-GC-ECD-ICP-MS combination developed by José Luis Gómez-Ariza and Tamara García-Barrera of Huelva University.

Their use of MHS-SPME avoided sample matrix, which constitutes the most frequent problem in analyzing oil samples. Their approach also achieved the determination of analytes under the established limits due to the high sensitivity of the ECD detector as well as the unequivocal identification by the element-specific ICP-MS detector.

Reference

[1] J.L. Gómez-Ariza, T. García-Barrera, J. Anal. At. Spectrom. 21 (2006) 884.

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Kohler wins award for doping control

The 2006 Manfred Donike Award for scientific excellence in doping control has been won by Maxie Kohler (Institute of Biochemistry, German Sport University, Cologne) for her research into the molecular mechanisms by which athletes metabolize 4-hydroxyandrostenedione and 4-hydroxytestosterone substances.

This annual award, sponsored by Agilent Technologies Inc. and first presented in 1997, recognizes distinguished scientific contributions in the field of sports medicine by scientists, who exemplify the spirit and the scientific leadership of doping-control pioneer Professor Manfred Donike and whose contributions significantly advance the cause of fairness in sports competition. The award comprises a medallion and a cash prize of €3500.

''Kohler's research represents an important step forward in understanding how athletes metabolize two frequently abused substances, enabling better detection of those substances during testing,'' said Stephen B. Crisp, international business development manager, Life Sciences and Chemical Analysis, Agilent. ''Her work is further proof that Prof. Donike's pioneering contributions and visionary leadership in developing anti-doping methods and technologies for international sporting competition continue to level the playing field for all athletes.''

Before assuming her present position at the German Sport University, Kohler received her master's degree in biology at Cologne University, where she gathered practical experience at the Anti-Doping Center using gas chromatography mass

Figure 3. Maxie Kohler (second from right), recipient of the 2006 Manfred Donike Award for scientific excellence in doping control. Also pictured (from left): Manfred Donike Doping Control Workshop organizer Wilhelm Schaenzer, Head, Cologne Anti-Doping Laboratory, Institute of Biochemistry, German Sport University, Cologne; Marie-Theres Donike, widow of Prof. Manfred Donike; and, Stephen B. Crisp, international business development manager, Life Sciences and Chemical Analysis, Agilent.

spectrometry (GC-MS) to analyze dietary supplements for the presence of anabolic steroids. Her work included the synthesis of reference compounds and metabolites of various substances used by athletes illegally to enhance performance; the foundation of her work focused on androstenedione and testosterone substances. She collaborated with other researchers from the Institute of Biochemistry at the German Sport University, including M.K. Parr, G. Opfermann and W. Schaenzer (Fig. 3).