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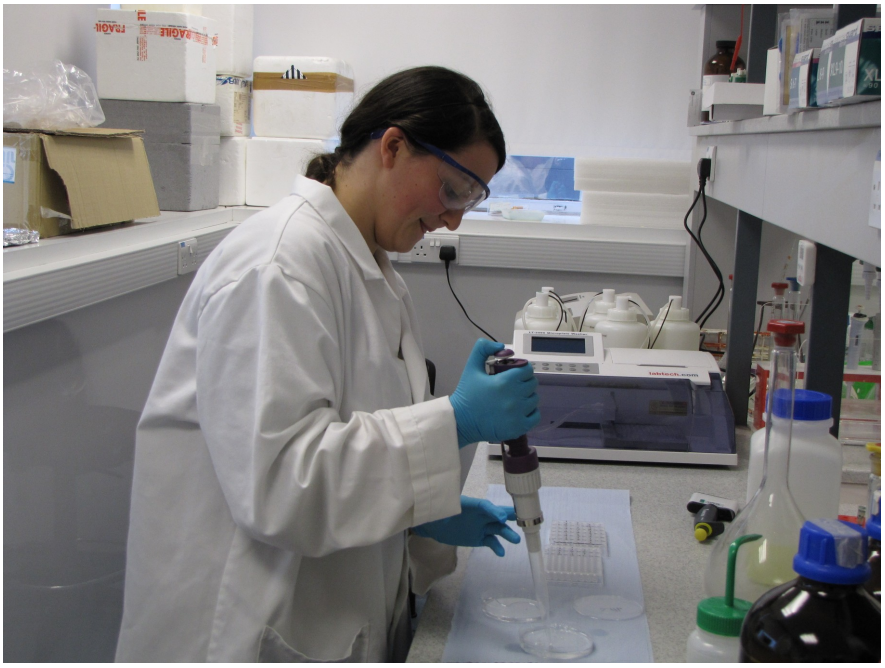
Biosensors, A Multidisciplinary Approach



ALVATEK

Monday 25th of June 2012

at

Bangor University
School of Chemistry
The Orton Lecture Theatre
Bangor University
LL57 2UW

RSC | Advancing the Chemical Sciences

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Foreword

Professor David Shepherd

Pro-Vice Chancellor for Research and Enterprise, Bangor University

I am delighted to welcome you to Bangor University, the host of this Royal Society of Chemistry Workshop on Biosensors. The workshop is funded by the Chemistry Biology Interface Division of the Royal Society of Chemistry and aims to highlight new research at the interface between biology and chemistry. The focus of this meeting is on Biosensors and seeks to highlight recent advances in this important and rapidly growing field of research. By bringing together local and international experts in the field it is hoped the event will generate new research collaborations and future funding. The meeting is open to researchers at all career stages and Masters and PhD students particularly encouraged to apply/attend with a poster session running throughout the meeting. The meeting is also supported by an international field of speakers from the University of Vienna, ICPF Prague University, Aberystwyth, Bangor, Cardiff, Manchester, a local company and funding bodies followed by a drinks reception.

I wish you all an exciting and profitable day.

David Shepherd

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Acknowledgements

Conference Organising Committee:

Dr Chris Gwenin, Tracey Roberts, Stevie Scanlan. With additional support from the administrative and technical staff and many of our postgraduate students.

Supported by the Chemistry Biology Interface Division of the Royal Society of Chemistry and by Steve Fryatt from Alvatek.

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Welcome to Bangor University

Opening its doors in 1884, the University was founded as the University College of North Wales as a direct result of a campaign in the late nineteenth century for higher education provision in Wales. An important feature of its foundation was the voluntary contributions made by local people, including farmers and quarrymen, from their weekly wages over a period of time. In 1893 the University of Wales, Bangor became one of the three original constituent colleges of the University of Wales. Today we have over 10,000 students and 2,000 members of staff.

The School of Chemistry at Bangor has a long and distinguished history over 125 years. It was one of the founding schools of the University College of North Wales (now Bangor University) and has a proud tradition of excellent chemists both working in and being trained at the School. The School of Chemistry offers a wide range of undergraduate courses, including its flagship MChem programmes. Research work in the school is of the highest quality and covers traditional areas of organic, inorganic and physical chemistry together with the newer areas of computational, environmental, materials and biological chemistry.

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Biosensors - A Multidisciplinary Approach

This workshop aims to highlight the novel research that is taking place at the interface between biology and chemistry; whilst enhancing the possibilities to network on a regional and international level. Biosensors are a growing field of research and the amalgamation of local and international experts within the field is hoped to generate new research collaborations and future funding applications. Masters and PhD students will be encouraged to apply/attend and a poster session will be held throughout the meeting.

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Programme

9.15 **Registration**

10.20 **Welcome**
Dr Christopher Gwenin, School of Chemistry, Bangor University
Professor David Shepherd, Pro-Vice Chancellor for Research and Enterprise, Bangor University

10:30 **The design of artificial recognition materials that can be utilized for sensor applications**
Prof Peter Lieberzeit, University of Vienna

11:00 **Developments towards ellipsometric readout of biochips**
Mr Matthew Gunn, and Dr Russell Morphew, from the group of Dr David Langstaff

11:30 **Towards the detection of TB**
Mr *Mark Pitts from the group of Dr Christopher. Gwenin*

12:00 **Decreased oxygen levels can affect the assessment of toxicity and genotoxicity in cell-based assays.**
Mr Christopher Addinsell from the group of Prof Richard Walmsley

12:30 **Lunch**
Discussion Groups

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14:00 **Funding Opportunities**
Short presentations

14:30 **Rapid Ammonia Detection: Only one part of the Jigsaw**
Dr Guido Drago,
Applied Enzyme Technologies

15:00 **Nanostructured functional thin films**
Prof Petr Kluson,
ICPF Prague University

15:30 **From transistors to photocontrol: engineering proteins for new applications and approaches**
Dr Dafydd Jones
Cardiff University

16:00 **Drinks Reception**

Speakers/Research groups

Professor Peter A. Lieberzeit

Prof. Peter Lieberzeit is a professor of analytical chemistry, and leads a working group for chemical sensors and optical molecular spectrometry.

Peters group actively participates in trans-European networks. During the last five years, it has successfully participated in an IP within EU-FP6 and currently is part of two project consortia in EU-FP7. As a specialty, it is also involved in EU-financed educational cooperation via the TEMPUS program. This internationality is also reflected within the group, whose current members combine an overall of seven different nationalities.

Some recent papers

Mustafa G, Hussain M, Iqbal N, Dickert FL, Lieberzeit PA, QCM Sensors based on Affinity Interactions between Organic Thiols and Molybdenum Disulfide Nanoparticles, Sens. *Actuators* B **162** (2012) 63-67.

Wangchareansak T, Sangma C, Choowongkomon K, Dickert FL, Lieberzeit PA, Surface molecular imprints of WGA lectin as artificial receptors for masssensitive binding studies, Anal. *Bioanal. Chem*. **400** (2011) 2499-2506.

Schirhagl R, Lieberzeit PA, Dickert FL, Chemosensors for Viruses Based on Artificial Immunoglobulin Copies, Adv. *Mater*. **22** (2010) 2078-2081

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Lieberzeit PA, Rehman A, Najafi B, Dickert FL, Real-life application of a QCM-based e-nose: quantitative characterization of different plant-degradation processes. Anal. *Bioanal.* Chem. **391** (2008) 2897-2903.

Patent

"Oil quality sensor" F. L. Dickert, P. Forth, P. Lieberzeit, G. Voigt, K. D. Marquardt, Intl. Pat. Appl. WO 98/19156, May 7th, 1998. US. Pat. 6,223,589, May 1st, 2001.

The design of artificial recognition materials that can be utilized for sensor applications

Abstract

Peter's group on Chemical Sensing and Rapid Analysis mainly focuses on the design of artificial recognition materials that can be utilized for sensor applications. The synthetic strategies include molecular imprinting into highly cross-linked polymers to address a range of analytes and contaminants ranging from small molecules up to entire microorganisms. Work at the moment extends towards generating "biomimicking" materials in the stricter sense of the word to allow for replacing natural systems by artificial ones. The second synthesis strategy includes the design of affinity materials in the form of nanoparticles for sensing volatile organic compounds and more recently composite materials with MIP to combine the better of two worlds. On the measuring side, the group applies a wide range of transducers, mainly mass-sensitive (quartz crystal microbalance), but also interdigital capacitors and different molecular spectrometry techniques, and also combines them to sensor arrays, where feasible.

Speakers/Research groups

Professor Manuel Grande

The Institute of Mathematics and Physics, which embraces two of the oldest and scientifically distinguished departments, Mathematics and Physics, was launched by Aberystwyth University in August 2003. Established shortly after the University of Wales was founded at Aberystwyth in 1872, these subjects were taught from the start and continue today with over 300 undergraduate and postgraduate students, including many from abroad.

Research in Applied Mathematics and Atmospheric Physics was well-established at Aberystwyth by 1900. In 1908 GA Schott predicted the phenomenon of synchrotron radiation at Aberystwyth almost 70 years before Julian Schwinger from Harvard won a Nobel Prize for developing the full theory. In 1940 EJ Williams, whose centenary was celebrated in 2003 in Llanwennog, discovered the pi-meson in Aberystwyth using one of the early cloud chambers. The pi-meson is one of the most important fundamental particles of the universe.

In more recent years Professor Ken Walters FRS established the internationally renowned Institute of Non-Newtonian Fluid Mechanics in which Applied Mathematics is directed at predicting the complex

behaviour of modern paints, polymers and foodstuffs. At the same time Physics at Aberystwyth has developed to include the physics of the Sun with the pioneering work of Professor Phil Williams on Space Weather. Amongst the most recent developments has been the foundation of the Materials Physics Laboratory, which is devoted to advancing glass and semiconductor Physics. This was officially opened by Wales' First Minister Rhodri Morgan in 2002.

Currently the Institute of Mathematics and Physics is initiating the Welsh Visualisation Centre, in conjunction with the WDA, IT companies and Objective 1. This £10m leading-edge project based at Aberystwyth will exploit state-of-the-art virtual reality technology for academic and industrial partnerships across Wales.

Developments towards ellipsometric readout of biochips

M. Gunn, R. Morphew, D. P. Langstaff

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Abstract

Ellipsometry is an optical technique which achieves very high sensitivity to surface properties by measuring the change in polarisation of reflected light. With the ability to rapidly detect and characterise a single monolayer on a surface, ellipsometry offers a fast and efficient method of studying protein – protein interactions. A feasibility study into the use of spectroscopic ellipsometry for the *in-situ* detection of Protein – Protein interactions demonstrated that an interaction between a Silicon wafer baited with a dilute solution of sGST, linked via gluteraldehyde, and an anti-sGST polyclonal antibody could easily be detected with the test system and the reaction kinetics determined. By using the Aberystwyth rapid imaging ellipsometer it is believed that biochip arrays with in excess of a hundred elements could be read out rapidly, simultaneously and *in situ* providing an efficient method of mass screening. Although tests so far have only involved a protein – antibody interaction, multi-layer biological complexes and non biological surface chemical processes could also be detected in this way.

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Speakers/Research groups

Dr. Chris Gwenin

Chris's Electrochemistry and Biosensors research group have several collaborations with industry and universities worldwide.

The group also includes many postgraduate students with their profiles available on the group website (http://electrochemistry-biosensors.eu.pn/) there are also visiting students on various exchange programmes; we are quite a big international family.

Patents:

C. D. Gwenin, M. Kalaji and P. A. Williams, **Improvements in and relating to biosensors**, Patent application WO 2005/056815 A1

V. V. Gwenin, C. D. Gwenin, and M. Kalaji, **Gold coated magnetic particles enabling nitroreductase delivery to cancer cells,** PCT/ EP2010/062871

Three patent application in conjunction with the detection of *M.tb.*

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Recent Papers:

C. E. French, and C. Gwenin, C. **Biosensors in bioprocess monitoring and control. In Fermentation Microbiology and Biotechnology**, 3rd edition, ed. El-Mansi, M., CRC Press, Boca Raton, in press

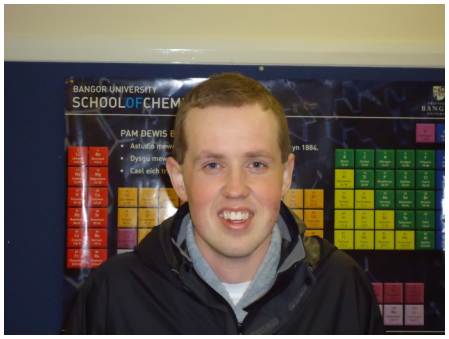
V. V. Gwenin, C. D. Gwenin and M. Kalaji, **Colloidal Gold Modified with a Genetically Engineered Nitroreductase: Towards a Novel Enzyme Delivery System for Cancer Prodrug Therapy.** *Langmuir* 27 14300 (2011) **DOI:** 10.1021/la202951p

M. Morozova, P. Kluson, J. Krysa, C. Gwenin, O. Solcova, **Oxalic acid sensors based on sol-gel nanostructured TiO_2 films**. *Journal of Sol-Gel Science and Technology*. 58 (1) 175 (2011) **DOI:** 10.1007/s10971-010-2374-7

M. P. Cude, and C. D. Gwenin, **Development of gold coated superparamagnetic iron oxide nanoparticles for nitroreductase delivery.** *ECS Trans* ECS Trans. **33**, 79 (2011) **DOI**: 10.1149/1.3583517

Towards the detection of TB

Mr Mark Pitts



Abstract

Tuberculosis is one of the leading causes of deaths worldwide even though it is both treatable and curable. All of the current diagnostic methods have been deemed either too long or unreliable by the World Health Organisation. A novel colour assay based on gold nanoparticles coated in mycolic acids and their sugar esters has been developed. The assay can distinguish TB positive and TB negative sera in only a few minutes without the need for any equipment to analyse the results.

I am currently a third year PhD student working on the development of a novel sensor for the detection of tuberculosis, under the supervision of Dr. C. Gwenin and Prof. M. Baird. Having previously completed an MChem degree at Bangor University, which included research projects on both sensors and mycolic acids, my current research gives me the opportunity to combine both these areas of study.

Speakers/Research groups

Prof Richard M Walmsley

The Walmsley Group

The group is located in Manchester. The laboratory accommodates the testing, production and development facilities of Gentronix Ltd, as well as PhD and other research projects. I have a long term interest in genome stability. This started with my post-doctoral work on telomeres, and after some work on genetic fingerprinting of yeasts in beer and nocosomial infections, I joined a yeast genome analysis consortium in which I set about hunting for new DNA repair genes. I developed a reporter assay to screen for knockout strains that had an altered DNA damage response. Validation of the assay led to development of the RAD54-GFP 'GreenScreen GC' genotoxicity assay, which underpinned the founding of Gentronix Ltd. Most recently, my focus has shifted to human cell systems. A PhD project lead to the development of a GFP reporter assay based on the promoter and other sequences from the human *GADD45a* gene: the 'GreenScreen HC' assay. It identifies all classes of genotoxic carcinogen and has a much higher specificity than any of the existing *in vitro* mammalian genotoxicity assays. It has now been used by over 100 companies, worldwide. The group has a continuing interest in the development of new and improved methods for the detection of genotoxicity, as well as

tackling the issues that afflict the field of genetic toxicology. Most recently, research projects have focussed on the investigation of particular groups of non-carcinogenic compounds which produce positive genotoxicity results in several different assays. One such example is discussed in this presentation.

Decreased oxygen levels can affect the assessment of toxicity and genotoxicity in cell-based assays.

Christopher Addinsell

Abstract

Abstract: The culture of mammalian cells is routinely carried out in high (5%) $C0_2$, but this only mirrors one aspect of the natural environment in the mammal. Whilst the air and $C0_2$ incubators have 21% oxygen, the level in the tissues is much lower, corresponding to an external level of

around 1- 5%. This means that cell lines are potentially exposed to greater oxidative stress than they evolved to tolerate. This project is testing the hypothesis that the relatively high levels of oxygen in the standard genotoxicity tests might contribute to the incidence of misleading positive results, particularly from antioxidant compound that become pro-oxidants. Dose response toxicity and genotoxicity data from the human B cell-derived lymphoblastoid cell line Tk6 will be presented from experiments carried out at different external Oxygen levels.

Speakers/Research groups

Dr Guido Drago

Profile:

Guido obtained his BSc in Physiology, MSc. in Forensic Science, and PhD. in Genetics at QMC, Kings College and UCL respectively. He carried out his postdoctoral training in Cardiac Biochemistry at the University of Leeds for 6 years, funded by the British Heart Foundation and the Royal Society and won a travelling scholarship to the University of Maryland in 1997.

In 1999 he joined Applied Enzyme Technology as a Research Officer and was made Managing Director in 2001. In 2005 he won a scholarship in Business Marketing Strategy at the Kellogg Graduate School of Management and completed a course in Business Enterprise & Development.

He currently runs a research group of 6 scientists dedicated to contracted research in the field of protein stabilisation, production of stabilised enzymes and the manufacture of biosensors. His current fields of interest include the identification of novel stabiliser molecules from natural sources and the development of novel biosensors for the environmental and agrifoods.

Rapid Ammonia Detection: Only one part of the Jigsaw

Abstract

Ammonia is one of the most widespread toxic pollutants in our rivers. It is a breakdown product of human and animal waste, and European and UK legislation imposes strict limits on its concentration in environmental waters. The Gwent Group of Companies (AET & GEM), delivered a handheld instrument which required the minimum of skill for operation, plus one shot disposable biosensors for the measurement of ammonia levels in water effluent. Over 2000 sensors were tested at the Starcross laboratory and showed good agreement with the lab based test carried out on a wide range of sewage effluent, river and marine based samples. What happened next? What did the market really want?

Speakers/Research groups

Dr. Peter Kluson, Associate Professor

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kluson@icpf.cas.cz; p.kluson@seznam.cz

Academy of Sciences of the Czech Republic / Institute of Chemical Process Fundamentals

Vice Chair of the Department of Catalysis and Reactor Engineering

Head of the NCFC Research Group (Nanotechnology, Catalysis, Fine Chemicals)

University of Jan Evangelista Purkyne

Head of the Photochemistry Group

Summary - *Author of 75 original papers, 2 monographies, 4 book chapters, 2 patents, 2 scientific series for the Czech Radio; H Index 11*

Brief CV

1983-1989 MSc. Degree at ICT Prague; **1990-1992** PhD at Department of Chem. Eng. SUNY Buffalo, USA, Certificate to Teach at US universities; **1995** Doctoral Thesis (PhD degree); **1996-1997** Teaching Assistant at ICT Prague; **1998** Post doctoral research fellow at Department of Chemistry University of Wales in Bangor, UK;

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1998/1999 Post doctoral research fellow at Imperial College in London, UK; **1999-2000** Visiting at Department of Chemistry, University of Wales in Bangor, UK; **2001-2002** Assistant Professor at Institute of Chemical Technology, ICT Prague; **2003** Habilitation, Associate Professor at ICT Prague; **2004** NCFC group established (Nanotechnology, Catalysis, Fine Chemicals); **2006** the NCFC group became a member of the Research Centre of Excellence for Nanosurface Engineering – NANOPIN; **2007** NCFC started to act as the hosting group for students for the Six Months Placement Scheme within the course MChem Course - CHEM 6008, Department of Chemistry, University of Southampton; Czech Chemical Society Annual Prize, Karl Preis Award 2007; **2008** NCFC Research Group transferred to ICPF of Academy of Sciences of the Czech Republic (www.icpf.cas.cz/hana); **2009** - Lectureship at University of J.E. Purkyně and at University of Pardubice, Chair of the national comittee of the Pannonian group of catalysis; **2010** - Scientific co-chair of the CHISA and EFCE joint conference (2000 participants), Centre of Photochemistry Research opened at University of J. E. Purkyne; Invited plenary speaker at SPEA 6 conference; **2011** – Guest Editor to Catalysis Today, the PF7 EU funded project PILGRIM completed.

Research areas: Nanotechnologies, Homogeneous and Heterogeneous Catalysis, Surface Science, Fine Chemistry, Photochemistry, Electrochemistry, Microporous Solids, Sol-gel Processes, DFT and mathematical modelling.

Nanostructured functional thin films

Abstract

Most of the thin layers practically employed as catalysts, adsorbents, sensors or electrodes reveal very low extent of characteristic (periodic) structural features. They are composed of arrays of pores of various shapes, sizes and volumes, mixed crystallographic phases, etc. and phenomena associated with their functions take place on surfaces containing physical (e.g. pits, ridges) and chemical (e.g. polar sites) defects. Due to availability of modern nanotechnology methods it is generally possible to produce very uniform, organized structures finalized as thin layers and exhibiting unique properties and functions. The lecture will focus on preparation of titanium (IV) oxide (titania) and other metal oxides` nanoparticulate thin films by a series of chemical, physico-chemical and physical methods including the sol-gel process carried out in the templating environment of lyotropic liquid crystals, its variation with ink-jet printing, Barrier-torch Discharge deposition, Magnetron Sputtering and the Modulated Hollow Cathode Plasma Jet Sputtering. The produced films differ in many structural features and also their photo-induced electrochemical characteristics are different. Thus the major topic treated in the lecture comprises the design of the feasible preparation methodologies yielding functional

thin layers in terms of the enhanced photo-induced electrochemical activity. It is, as it will be shown, the inherent function of the specific structural properties. The produced layers are thoroughly described by means of a series of characterization techniques including atomic force microscopy (AFM), scanning electron microscopy (SEM), X-ray diffraction (XRD), X-ray photoelectron spectroscopy (XPS), ultraviolet-visible spectroscopy (UV-vis), Fourrier transformed infrared spectroscopy (FTIR), Raman spectroscopy, ellipsometry, physical adsorption, profilometry and surface wettability. The films` functional features are clearly demonstrated on various tests, including voltammetry, and amperometry. The new concept of invisible bar codes is described together with the example of a sensor for oxalic acid. Other application strategies towards biosensors are also outlined.

Speakers/Research groups

Dr Dafydd Jones

School of Biosciences, Cardiff University, Cardiff.

The main focus of the Jones research group is exploring the structural and functional plasticity of proteins, our research involves the study of specific protein systems (e.g. subtilisin proteases), conformational switching (e.g. protein misfolding), construction of artificial protein scaffolds (e.g. novel molecular switches), synthetic biology (e.g. expanded genetic code) and protein nanocomponents (e.g. single molecule bioelectronics). Both rational protein engineering and directed evolution are used to create new proteins and standard biochemical approaches together with structural biology, molecular dynamics, biophysics and single molecule microscopy are used to investigate the properties of these novel proteins. We have also recently developed several transposon-based methods for the directed evolution of proteins using non-homologous recombination.

Recent selected publications.

Jones, DD (2011) Recombining Low Homology, Functionally Rich Regions of Bacterial Subtilisins by Combinatorial Fragment Exchange. *PLoS One* **6,** e24319

Della Pia EA, Elliott M[a], Jones DD[a], & Macdonald JE (2012) Orientation-Dependent electron transport in a single redox protein. *ACS Nano* **6**, 355-61. [a] joint corresponding authors.

Gamble M, Kunze G, Dodson EJ, Wilson KS & Jones DD. (2011) Regulation of an intracellular subtilisin protease activity. *Proc Natl Acad Sci* **108**, 3536-41.

Della Pia, E., Chi, Q[a], Jones, DD[a], Macdonald, EJ, Ulstrup, J & Elliot, M (2010) Single-molecule mapping of long-range electron transport for a cytochrome b_{562} variant. *Nano Letters* **11**, 176-82. [a] joint corresponding authors.

Edwards WR, Williams AJ, Morris JL, Allemann RK & Jones DD (2010) Regulation of b-lactamase activity by remote binding of heme: functional coupling of unrelated proteins through domain insertion. *Biochemistry* **49**, 6541-9.

Vévodová J, Gamble M, Künze G, Ariza A, Dodson E, Jones DD[a] & Wilson KS[a]. (2010). Crystal structure of an intracellular subtilisin reveals novel structural features unique to this subtilisin family. *Structure* **18**, 744-55. [a] joint corresponding authors.

Stott KM, Yusof AM, Perham RN & **Jones DD** (2009). A surface loop directs conformational switching of a lipoyl domain between a folded and a novel misfolded structure. *Structure* **17,** 1117-27.**From transistors to photocontrol: engineering proteins for new applications and approaches**

Abstract

Eduardo Della Pia[1,2], James Arpino[1], Sam Reddington[1,3], Eric Tippmann[3], Martion Elliot[2], Emyr Macdonald[2] and Dafydd Jones[1].
[1] School of Biosciences, [2] School of Physics and Astronomy & [3] School of Chemistry, Cardiff University.

Central to synthetic biology is the ability to construct new protein components with desired functionality that may not exist in the natural protein repertoire. Simple but effective protein engineering approaches can be very useful, generating protein derivatives for use in synthetic biology and bionanotechnology. For example, an electron transfer protein has been rationally engineered to bridge conducting surfaces; regulation of current flow at the single molecule level was observed, demonstrating potential for use in molecular electronics. To introduce radically new functionality into existing protein templates, new protein engineering approaches can sample an expanded sequence space range or even new chemistry. For example, new protein scaffolds have been constructed through a directed evolution domain insertion approach that act as protein switches and energy transfer systems by marrying the activities of normally disparate

proteins. Construction of artificial protein switches that change their properties in response to a desired signal open new possibilities for creating novel cellular modulators and biosensors. Targeted proteins can also be evolved to incorporate unnatural amino acids using modified cellular protein synthesis systems. Chemistry inherent in the non-natural amino acid will allow proteins to sample physicochemical properties not available from within the 20 natural amino acid set and will thus allow the introduction of distinct new functionality. We have successfully utilised photosensitive non-natural amino acids to control with high spatial and temporal resolution the activity of proteins using light.

Notes

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