**Institution: Durham University**

**Unit of Assessment: 8**

**Title of case study: Lanthanide complexes in analysis and diagnosis**

## 1. Summary of the impact

This case describes the impact of new emissive metal complexes, the development of associated optical equipment and knowledge dissemination based on lanthanide chemistry. Luminescent lanthanide complexes have been commercialised for drug-screening assays (France, USA); responsive probes have been developed to measure bicarbonate, citrate, lactate and urate in bio-fluids using ratiometric analysis of metal-based emission; and clinical studies are underway assessing the use of citrate as a metabolic marker for prostate cancer. Knowledge developed in Durham has led to expert witness work that has helped determine the outcome of hundreds of litigation cases. The first open settlement in the USA was $5 million; and there have been scores of undisclosed settlements. This has contributed to new FDA guidance that reduced the sales of one problematic gadolinium contrast agent by $90 million in the USA alone. Finally, a related "Spectroscopy in a Suitcase" outreach project was launched for UK schoolchildren and shown to over 5000 pupils, helping them link our basic research to societal impact.

## 2. Underpinning research

The rare earth elements are ubiquitous in modern society. A mobile phone contains 9 different lanthanide elements (ca 120 mg), and they are widely used in security printing (Eu) and as phosphors in displays (Eu, Tb). Since 1993, Durham research led by Parker (Durham staff 1982–present) and Beeby (Durham staff 1992–present) has addressed the synthesis, structure, mechanism of action and function of lanthanide coordination complexes [1-7]. Applications range from highly emissive lanthanide complexes to paramagnetic complexes of Gd used as contrast agents for MRI. Parker, for example, served as a consultant (1994–2007) for the contrast agent companies, Guerbet (Paris) and Bracco (Milan), and Beeby worked closely with Horiba Jobin-Yvon on instrumentation development (e.g. integrating sphere for quantum yield determinations) to help understand these materials.

Luminescent complexes of Eu and Tb whose emission profile is a sensitive function of the local pH, $pO_2$ or concentration of key bioactive anions or macromolecules were created in Durham between 1998 & 2011 [1,2]. These typically work via reversible anion binding to the metal centre displacing water and changing the spectral form and relative intensity of the emitted light. Ion affinity and selectivity can be controlled by varying the effective charge and steric demand at the metal centre. Using a mixture of Eu and Tb complexes, or by analysing the intensity ratio of two well-separated bands in the Eu emission spectrum, ratiometric analyses are enabled, allowing accurate calibration (WO 2008/007089, granted 2010 in Europe: EP 2041571 B1; US patent 8,501,398 (2013)). This has allowed optical sensors to be devised that can be calibrated to measure directly bicarbonate, urate, lactate and citrate in a variety of bio-fluids including serum, urine and seminal fluid (original filing by Durham University for citrate: US patent 8,193,174 granted 05.06.2012; for citrate and lactate: WO 2010/086615 A1 published in USA as US 2011/0287558A1 and in Europe as EP 2391610 A0).

Factors that limit the optical brightness of these probes have also been studied, especially processes that quench the excited states along the photochemical pathway [4]. For example, details of the quenching of the ligand and metal-based excited states by energy and electron transfer processes were examined, using time-resolved spectroscopic methods. Between 2005 and 2011 the research was extended beyond *in vitro* work, by developing spectral imaging in optical microscopy of pH and anion-induced changes *in cellulo*, paving the way for applications in cell biology. New portable optical instrumentation has been developed by Beeby and Dr Robert Pal to measure the optical signal selectively. A hand-held, time-gated emission spectrometer is being developed, in partnership with Ocean Optics (USA) [7]. Ethical approval was given in November 2009 for clinical studies to begin assessing citrate as a screening marker in prostate cancer.

Parker has also been developing the chemistry of contrast agents for MRI since 1991, creating new families of kinetically stable macrocyclic complexes that resist premature dissociation of the metal ion in vivo. [8] In addition, he has been creating new series of very bright Eu and Tb complexes [5,6], designing new sensitising moieties, such as azaxanthones/azathiaxanthones and

with Olivier Maury (Lyon) and CISbio, he has developed Eu complexes with a brightness of the same order as that of green fluorescent protein [5].

**3. References to the research**


[1] J. I. Bruce, R. S. Dickins, T. Gunnlaugsson, S. Lopinski, M. P. Lowe, D. Parker, R. D. Peacock, J. J. B. Perry, S. Aime and M. Botta, "The selectivity of reversible oxy-anion binding in aqueous solution at a europium and terbium centre; signalling of carbonate chelation by changes in the form and circular polarisation of luminescence emission", *J. Am. Chem. Soc.*, 2000, **122**, 9674–9684. **DOI**: 10.1021/ja001797x. **[193 citations]**

[2] R. S. Dickins, J. A. K. Howard, A. S. Batsanov, M. Botta, J. I. Bruce, C. S. Love, D. Parker, R. D. Peacock, and H. Puschmann, "Structural, luminescence and NMR studies of the reversible binding of acetate, lactate, citrate and selected amino-acids to chiral di-aqua ytterbium and europium complexes", *J. Am. Chem. Soc*., 2002, **124**, 12697–12705. **DOI**: 10.1021/ja020836x. **[160]**

[3] A. Beeby, I.M. Clarkson, R.S. Dickins, S. Faulkner, D. Parker, L. Royle, A.S. de Sousa, J.A.G. Williams and M. Woods, "Non-radiative deactivation of the excited states of europium, terbium and ytterbium complexes by energy matched OH, NH and CH oscillators: an improved luminescence method for establishing solution hydration states", *J. Chem. Soc., Perkin Trans*. *2*, 1999, 493–504. **DOI**: 10.1039/A808692C. **[670]**

[4] R. Pal, L. C. Costello and D. Parker, "A europium luminescence assay of lactate and citrate in biological fluids", *Org. Biomol. Chem*. 2009, **7**, 1525-1528. **DOI**: 10.1039/b901251f. **[55]**

[5] J. W. Walton, M. Soulie, A. Bourdolle, S. J. Butler, M. Delbianco, L. Lamarque, C. Andraud O. Maury, B. K. McMahon, R. Pal, H. Puschmann, J. M. Zwier and D. Parker, Very bright europium complexes that stain cellular mitochondria, *Chem. Commun*. 2013, **49**, 1600–1602. **DOI**:10.1039/C2CC35247H. **[6]**

[6] P.A. Atkinson, K.S. Findlay, F. Kielar, R. Pal, D. Parker, R.A. Poole, H. Puschmann, P.A. Stenson, A.L. Thompson and J. Yu, "Azaxanthones and azathiaxanthones as sensitisers for europium and terbium luminescence", *Org. Biomol. Chem.*, 2006, **4**, 1707–1722. **DOI** 10.1039/B601357K. **[37]**

[7] R. Pal, A. Beeby and D. Parker, "Rapid and accurate analysis of citrate in low-volume seminal fluid samples using a time-gated and ratiometric measurement of europium luminescence", *J. Pharmaceut. Biomed. Anal.* 2011, **56**, 352–358. **DOI**:10.1016/j.jpba.2011.05.023. **[12]**

[8] K. P. Pulukkody, T. J. Norman, D. Parker, L. Royle and C. J. Broan, "Synthesis of charged and uncharged complexes of gadolinium and yttrium with cyclic polyazaphosphinic acid ligands for *in vivo* applications", *J. Chem. Soc. Perkin Trans 2,* 1993, 605–620. **DOI**: 10.1039/P29930000605. **[72]**


The work described was supported by successive EPSRC grants to Parker over the period 1998-2011 including: GR/M04594, £173K, 1998–2001; GR/S15952, £94K, 2004–7; EP/D061873, £281K, 2006–2009; EP/I010319/1, £642K, 2011–14. Industrial funding includes ongoing support from CISbio Bioassays, Codolet, France of £415K, 2005-2014. EU support included invited participation in two EC Networks of Excellence: EMIL (2004-9) £47K and DIMI (2005-10) £215K. Parker also won an ERC Advanced Investigator Grant of €2.5M (FCC-266804; 2011-16) on "Functional Coordination Chemistry" to support research in metal complex systems and develop mechanistic understanding and exploitation of function.

The academic research quality and international esteem is attested by 23 named, plenary and keynote lectures on this topic in the period, various awards (e.g. 2011/12, RSC Ludwig Mond medal; 2012 triennial Lecoq de Boisbaudran Award in rare earth science) and invited consultancy work for commercial companies (CISbio; Molecular Devices; Guerbet, Bracco s.a.). Parker was awarded an FRS in 2002 based in part on his contributions in this area of science. He was also appointed as an expert witness on lanthanide chemistry for international patent litigation cases (2005 Taylor-Wessing London; 2003 Two Birds London) and for a major on-going multi-district litigation (2008 to present) for Spangenberg-Shibley and Liber (Cleveland, USA).

**4. Details of the impact**

Following the first publications on emissive lanthanide complexes in the late 1990s, several

companies independently contacted the Durham chemists and began to support research in Durham. These included: Sensors for Medicine and Science (1999–2001, Gaithersburg USA; Parker became a foundation shareholder); Molecular Devices (2004–7, Sunnyvale, California); and CISbio Bioassays (2005–2014, Codolet, France). In 2007/8, Molecular Devices marketed an assay [Im1] for high throughput screening of kinase inhibitors using a nanoparticle labelled with a Tb complex developed in Durham [6], a five-figure deal was agreed with the University, and it was protected by a primary patent (US Patent 7,517,701, 3 named Durham inventors) [Im1]. Consecutive direct grant support from the French firm CISbio developed new bright Eu complexes for time-resolved assays protected by 4 patents (most importantly: "Nouveaux agents complexants et complexes de lanthanide correspondant et leur utilisation comme marquers luminescents", French Pat. No. 1000118536; filed January 2013). These products, based on Durham research [5], are being considered for commercialisation [Im3] as potential replacements for the emissive Eu cryptate complexes used since the mid-1990s in high throughput, time-resolved assays for the pharmaceutical industry.

A Durham University spin-out company FScan Ltd [Im2] (company number 6550089), was set up in 2009 to examine the use of various anions as screening biomarkers using responsive luminescent probes. Initial support was via the EPSRC follow-on-fund (EP/G004773; £99K) and subsequent support was raised from the regional NStar Finance company (£190K), the Northern Universities N8 METRC fund (£50K), and Ocean Optics Inc. ($10K). Granted patents include EP 2041571 B1 and US patent 8193174.

The FScan-related research led to a test of Durham complexes in screening men for prostate cancer, by measuring citrate levels initially in prostatic then in seminal fluid samples (1 microlitre only needed) [4,7]. Ethical approval in late 2009 allowed an NHS study of 60 patients to be undertaken in 2010/2011 with the James Cook University Hospital, Teesside [Im4]. This showed that a distinction could be made between patients with high or intermediate grade prostate cancer from those with low-grade cancer or benign hyperplasia. The first UCLH trial (Mark Emberton, clinical lead, 240 patients) is underway, 80 samples have been measured (Oct. 2013) and it will compare citrate screening with more expensive methods such as those based on MRI and MRSI.

International publicity in 2009 [Im5] for the citrate test for prostate cancer raised significant public & commercial awareness. The story was carried by Reuters and reported in over 50 countries and in at least 30 magazines and papers (e.g. as a front page lead in the Daily Express; page 3 of the Sun; also in the Hindu in India). Live interviews were given on 5 radio stations in the UK and Eire and it was covered in Channel 4 News and on the BBC web site.


**Figure 1. Publicity and science behind the FSCan prostate cancer diagnostic test and FScan's Robert Pal undertaking related outreach demonstrations.**

The optical instrumentation developed in this work has also been incorporated into the "Spectroscopy in a Suitcase" outreach project. This allows the distribution of portable spectroscopy equipment to schools throughout the UK. Since September 2010, over 5000 pupils and members of the public have been educated and entertained by 35 on-site demonstrations by a team led by Dr A Beeby. Examples include demonstrations of Eu emission (e.g. on the €10 note) following UV excitation, and the biomedical applications of emissive complexes are explained to attendees.

Durham's research-derived expertise in lanthanide coordination chemistry has also had major impact in the healthcare arena, particularly through changes in FDA regulations surrounding Gd contrast agents used in millions of MRI scans every year. This arose from the key scientific arguments and insight Parker provided between 2008 & 2013 [Im6,8] to Cleveland lawyers developing the plaintiffs' case in a multi-district litigation around the recently defined disease,

nephrogenic systemic fibrosis (NSF).

In 1993, Parker stated [8] that macrocyclic contrast agents, "are more kinetically stable *in vivo* than DTPA-based ligands, and should avert long term (i.e. chronic, rather than acute) toxicity problems". These concerns proved prophetic. In 2005/6, the fatal condition, nephrogenic systemic fibrosis emerged, mostly in diseased renal patients. A link was made to the "unsafe" use of DTPA-based Gd contrast agents that are susceptible to acid promoted dissociation, particularly GE Healthcare's Omniscan and the related Optimark product.

Durham advised the Ohio law firm involved via an initial 12 page report followed by a formal court deposition involving 8 hours of video/oral defence in London in September 2010. On January 24 2011 GE Healthcare acknowledged these arguments and settled the first and four subsequent "bellwether trials" out of Court; each was linked to scores of cases with undisclosed settlements [Im 6,7]. Parker continued to advise the prosecution for the first case involving a single individual to come to Court in March 2013. The Court asked GE Healthcare to pay $5M to Mr Paul Decker, who had been rendered an invalid following one Omniscan MRI scan in 2005 [Im7,8].

In December 2009, the FDA issued new guidelines to the radiology community advising against the use of DTPA based systems in renal patients. This minimised risk to the 40 million patients each year that undergo a Gd-assisted MRI scan. Since its launch, the DTPA-diamide contrast agent Omniscan has been used in over 50 million MRI procedures at a cost of around $30 per scan. Omniscan's share of the Gd contrast agent market fell from 32% in 2005-7 (ca $130M) to just 10% in 2012. Macrocyclic contrast agents are likely to dominate this market in future.

**5. Sources to corroborate the impact**


[Im1] Molecular Devices assay: www.moleculardevices.com. US Patent 7517701 (granted April 2009) contains details of IMAP assay method; independent validation is in *Nature Protocols*, 2008, **3**, 1350. The former Vice President of Molecular Devices oversaw commercial development of the IMPA assay and will confirm use of the Durham Tb complex.

[Im2] FScan company: www.fscanltd.co.uk UK registered company 6550089, 31 March 2009. FSCan hold 4 patents.

[Im3] CISbio high throughput arrays: www.cisbio.com Contact CISbio (gmathis@cisbio.com) for corroboration of the status of emissive Eu complexes bring developed.

[Im4] Clinical trials: details of the pilot clinical work at the James Cook University Hospital that recruited patients to assess the scope and sensitivity/specificity of citrate as a metabolic marker in seminal fluid can be traced via: www.cancerhelp.org.uk/trials/trials-search/study-looking-new-test-diagnose-prostate-cancer-measuring-citrate

[Im5] Prostate screening: Details of publicity associated with the 2009 press release (syndicated worldwide by Associated Press and Reuters) can be traced via: www.nhs.uk/news/2009/05May/Pages/3MinuteProstateCancerTest.aspx This links to press articles published (*inter alia*) in the Daily Express (front page lead), Sun, Hindu, Daily Mail, Daily Telegraph, Men's Health, BBC News, Channel 4 www sites (>500,000 hits).

[Im6] Law case settlement: details of the first out of court GE Healthcare settlement presided over by Judge Dan Polster in the Ohio Court on January 24 2011 can be traced via: http://gadoliniumlawsuit-info.com/2011/03/judge-dan-polster-key-ruling-gadolinium-lawsuit/ For updates see: http://gadoliniumlawsuit-info.com/2011/05/gadolinium-multidistrict-litigation-lawsuits-settlements Details of the amended FDA guidance (09/12/09) following realisation of the NSF problem can be traced at: http://www.fda.gov/drugs/drugsafety/ucm223966.htm

[Im7] Trial settlement: details of the first case to proceed to trial, completing on March 22 2013, can be found at: http://finance.yahoo.com/news/ashcraft-gerel-llp-jury-orders-160500877.html The story is related in the Washington Post at http://washpost.bloomberg.com/Story?docId=1376-MK2PHO6S972W01-4NJQJIG0GSPKRHIAN399NB7SBS

[Im8] Expert witness contributions: a signed letter from the Cleveland law firm Spangenburg, Liber and Shipley attests to the key role played by Parker in developing the scientific case for the prosecution in these litigation cases.