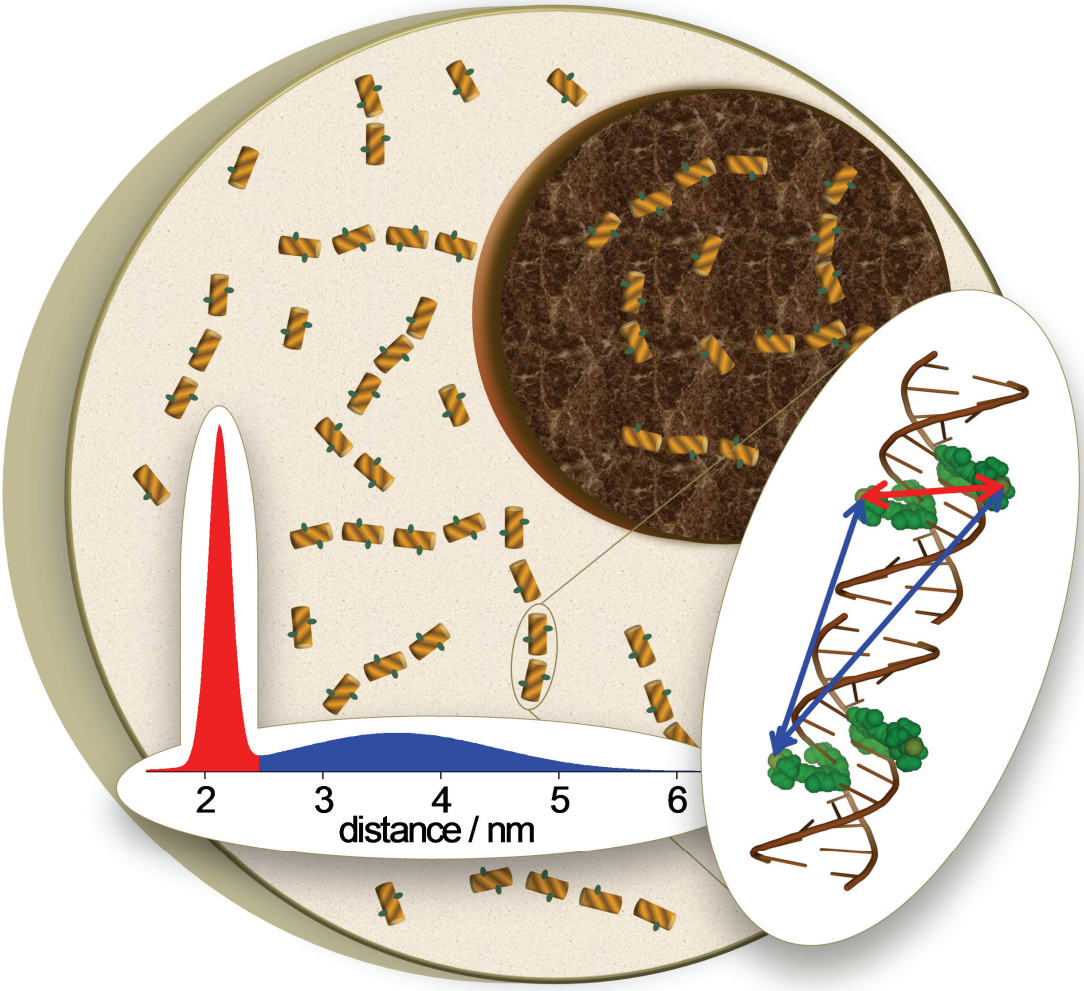
The 44th Annual International Meeting

of the

ESR Spectroscopy Group

of the

Royal Society of Chemistry



University of York

3rd – 7th April 2011

Contents

Conference Programme

Sunday 3rd April		
16.00 – 18.30	Registration	NSLC Atrium
18.30 – 20.00	Dinner	NSLC Restaurant
20.00 – 22.30	RSC Wine Reception and free bar	NSLC Atrium

Monday 4th April		
07.30 – 08.55	Breakfast	NSLC Restaurant
Session 1 Chair: Damien Murphy		
08.55 – 09.00	Mark Newton	Conference opening and welcome note
09.00 – 09.30	Victor Chechik	**Keynote Lecture:** Using spin traps in heterogeneous reactions
09.35 – 09.50	Daniel Cardoso	Combined ST/EPR and LC-MS approach to identify spin adducts and investigate the reactivity of beer bitter acids towards 1-hydroxyethyl radical
09.55 – 10.10	Klaus Stolze	Synthesis of different 5-alkoxycarbonyl-4-hydroxymethyl-5-methylpyrroline *N*-oxide derivatives
10.15 – 10.30	Phil Barker	An ESR method to determine relative volatility of HALS in surface coatings
10.35 – 11.05	Tea & Coffee	NSLC Atrium
Session 2 Chair: Helen Williams		
11.05 – 11.25	Snorri Sigurdsson	**Invited Lecture**: Non-covalent and site-directed spin labeling of nucleic acids
11.30 – 11.45	Elisabetta Mileo	Characterization of supramolecular systems by EPR spectroscopy
11.50 – 12.05	Dennis Kurzbach	Spin-Probing Nanoscopic Inhomogenities in Pluronics
12.10 – 12.25	René Boeré	An electrochemical and EPR spectroelectrochemical study of *peri*-substituted naphthalenes
12.30 – 14.00	Lunch	Derwent Dining Hall
Session 3 Chair: Chris Kay		
14.00 – 14.15	Ivan Krstić	**Jeol Student Prize Talk:** In-cell PELDOR on Nucleic Acids
14.20 – 14.35	Petra Lüders	**Jeol Student Prize Talk:** Distance Measurements on Lanthanide Ion – Nitroxide Radical Spin Pairs in the Nanometre Range by Relaxation Enhancement
14.40 – 14.55	Maxie Roessler	**Jeol Student Prize Talk:** A Single Supernumerary Cysteine Adjacent to the Proximal Fe-S Cluster Confers Oxygen Tolerance on a [NiFe]-Hydrogenase
15.00 – 16.30	Tea & Coffee: **Posters (EVEN)**	NSLC Atrium
Session 4 Chair: Rachel Haywood		
16.30 – 16.50	Hassane Mchaourab	**Invited Lecture**: Protein conformational dynamics from spin labeling EPR spectroscopy
16.55 – 17.10	Matt Bawn	Myoglobin and nitrite: an EPR investigation of a physiologically relevant new role
17.15 – 17.30	Dimitri Svistunenko	The catalatic mode of *Mycobacterium tuberculosis* catalase-peroxidase as studied at different pH values
17.35 – 17.50	Sergei Dzuba	Dynamics and topology of spin-labeled lipids and peptide antibiotics in model phospholipids membranes probed by stimulated ESE
17.55 – 18.55	Stefan Stoll	EasySpin
19.00 – 20.30	Dinner	Derwent Dining Hall
20.30 – 24.00	JEOL Reception and free bar	NSLC Atrium

Tuesday 5th April		
07.30 – 09.00	Breakfast	NSLC Restaurant
Session 5 Chair: Christiane Timmel		
09.00 – 09.30	Stephen Hill	**Keynote Lecture:** Molecular Magnetism and Multi-High-Frequency EPR at the National High Magnetic Field Laboratory
09.35 – 09.50	Graham Smith	Can high field PELDOR be used as a standard biophysical tool to extract quantitative orientational information in SDSL studies?
09.55 – 10.10	Deborah Mitchell	X-Band Rapid-scan EPR
10.15 – 10.30	Mark Newton	The temperature dependence of the DNP enhancement of water protons at 3.4 Tesla
10.35 – 11.05	Tea & Coffee	NSLC Atrium
Session 6 Chair: Ilya Kuprov		
11.05 – 11.25	Eric McInnes	**Invited Lecture:** A spectroscopic study of exchange-coupled orbitally-degenerate ions: six-coordinate cobalt(II) dimers.
11.30 – 11.45	Lorenzo Sorace	Magnetic anisotropy in propeller-like Single-Molecule Magnets: insights from HF-EPR
11.50 – 12.05	Stephen Sproules	Beguiling Crystallography Cured by EPR: Rhenium Dimers with Multiple Metal Bonds
12.10 – 12.25	Ozlen Erdem	Advanced pulsed EPR investigation of model complexes of the [FeFe] hydrogenase active site: bulky bridges *vs* bulky ligands
12.30 – 14.00	Lunch	Derwent Dining Hall
14.00 – 18.00	Free afternoon and walking tour around York	Buses into York city centre leave at 14.00; walking tour is 14.30-15.30.
18.00 – 19.30	Dinner	Derwent Dining Hall
Session 7 Chair: Mark Newton		
19.30 – 21.00	Thomas Prisner	**Bruker Lecture:** New developments in EPR and DNP and application to biomolecular research
21.00 – 24.00	Bruker Reception and free bar	NSLC Atrium

Wednesday 6th April		
07.30 – 09.00	Breakfast	NSLC Restaurant
Session 8 Chair: Fraser MacMillan		
09.00 – 09.30	Wayne Hubbell	**Keynote Lecture:** Exploring the energy landscape and dynamic modes of a protein with site directed spin labelling
09.35 – 09.50	Johann Klare	Site-directed spin labelling EPR – targeting protein structure, dynamics and function
09.55 – 10.10	David Norman	A Site Specific Spin Labeling and EPR Study of a complex between Endonuclease I and DNA-4-way junction
10.15 – 10.30	Yuri Tsvetkov	PELDOR applications for the spatial distribution studies of paramagnetic centers
10.35 – 11.05	Tea & Coffee	NSLC Atrium
Session 9 Chair: David Norman		
11.05 – 11.25	Howard Halpern	**Invited Lecture:** Spectral information from unexpected places: tumor physiology with in vivo EPR imaging
11.30 – 11.45	Gunnar Reginsson	PELDOR measurements on nitroxide biradicals and non-covalently spin labelled DNA using Hiper
11.50 – 12.05	Valérie Belle	Viewing the Human Pancreatic Lipase lid opening by Site Directed Spin Labeling combined with CW and pulsed EPR spectroscopy
12.10 – 12.25	Katharina Pirker	Polymerisation mechanism at the outer membrane usher of Type 1 pili from *E.coli* investigated by SDSL-EPR
12.30 – 14.00	Lunch	Derwent Dining Hall
Session 10 Chair: Eric McInnes		
14.00 – 14.15	Vasily Oganesyan	MD simulation of spin labelled yeast iso-1 cytochrome *c* protein: a tool for predicting the EPR
14.20 – 14.35	Ilya Kuprov	*Spinach* – a software library for simulation of spin dynamics in large spin systems
14.40 – 14.55	Stephanie Simmons and Vasileia Filidou	Spin Ensemble Entanglement
15.00 – 15.15	Christopher Wedge	Spin Dynamics in Molecular Magnets
15.20 – 16.15	Tea & Coffee: **Posters (ODDS)**	NSLC Atrium
Session 11 Chair: Victor Chechik		
16.15 – 16.35	Michael Davies	**Invited Lecture**: Radical transfer reactions mediated by heme proteins and their prevention
16.40 – 16.55	Rachel Haywood	Investigating UVA-induced DNA-photosensitiser radical combinations in DNA-riboflavin; and relevance to cell nuclei
17.00 – 17.15	Helen Williams	Applications of EPR Spectroscopy in Pharmaceutical Analysis
17.20 – 17.35	Mohamed Morsy	EPR/Alanine Dosimetry: Final Chapter?
17.40 – 18.10	AGM ESR Group RSC	NSLC Lecture Theatre (all welcome to attend)
19.30 – 22.30	Banquet	Merchant Adventurers Hall (**buses leave at 19.00**)

Thursday 7th April		
07.30 – 09.00	Breakfast	NSLC Restaurant
Session 12 Chair: Dimitri Svistunenko		
09.00 – 09.30	David Collison	**Keynote Lecture:** Electronic structure of large polymetallic molecules: a multi-frequency EPR approach
09.35 – 9.50	Emma Carter	An EPR Investigation of non-C_2 Symmetric Cu(Salen) based Complexes
9.55 – 10.10	Christian Teutloff	EPR investigations on mutants of the membrane-bound [Ni-Fe]-hydrogenase from *Ralstonia eutropha*
10.15 – 10.30	Jeffrey Harmer	HYSCORE and ENDOR spectroscopy to determine the g-matrix orientation of the reduced [2Fe-2S] cluster in palustrisredoxin
10.35 – 11.05	Tea & Coffee	NSLC Atrium
Session 13 Chair: David Collison		
11.05 – 11.25	Stefan Weber	**Invited Lecture**: Origin of Light-Induced Spin-Correlated Radical Pairs in Cryptochromes and Related Blue-Light Active Proteins
11.30 – 11.45	Stefan Stoll	Tryptophan radicals
11.50 – 12.05	Maryam Shabestari	Alzheimer amyloid peptide aggregation studied by SL-EPR
12.10 – 12.25	Günter Grampp	ESR-spectroscopy in ionic liquids: Investigations on the dynamics and kinetics of organic radicals
12.30 – 14.00	Lunch	Derwent Dining Hall
CONFERENCE END - DEPARTURE		

Information for delegates

Getting there.

You will need to get a bus or a taxi from the York railway station to the campus, as the University is two miles away in the village of Heslington. This can be achieved by using either the First ftr bus service (service no. 4), or the York Pullman unibus service (service no. 44). The journey time is only 15 minutes, and the service runs every 15 minutes. You need the stop for Morrell Library, University of York. The walking route from the bus stop to the conference venue is shown on the campus map below. A journey by taxi from the railway station to the University will take approximately 15 minutes (cost around £7). There is a taxi rank just outside the station. You should ask the driver to take you to the Alcuin College at the University of York (to collect accommodation keys) or Campus North car park (conference registration desk).

How to find:

- **Registration**

Registration will be in **National Science Learning Centre** (**NSLC**, see map below). When you arrive, either go to registration, or first visit Alcuin reception to collect the keys for accommodation (available after 14:00). Please wear your conference badge **at all times** (including lunches/dinners).

- **Accommodation**

All single rooms are in Alcuin college (see map below). Double rooms are in Franklin house. The keys can be collected from the Alcuin reception after 14:00 (the reception is open 24 h). If you arrive earlier, you will be able to store luggage in NSLC (ask at NSLC reception).

- **Lectures and posters**

All lectures will be in NSLC Lecture theatre 1. Posters and exhibits will be in NSLC Atrium.

- **Coffee breaks and receptions**

All coffee breaks and receptions will be in NSLC Atrium (where the posters are). Free bar will be in NSLC restaurant.

- **Meals**

Dinner on Sunday and all breakfasts will be in NSLC restaurant. Lunches and dinners on Mon-Thu will be in Derwent dining hall (see map below).

- **Banquet**

Banquet will be held in Merchant Adventurers Hall on Wednesday at 19.30-22.30 (see York city centre map). The buses will leave from the Alcuin reception (see campus map) at 19.00.

- **Shops**

A convenience store (Costcutter) is not far from the NSLC (in Market Square across the University Road). It is closed on Sunday but open 8am-6pm Mon-Thu. There are cash points near Costcutter. Heslington village (bottom right on the campus map) has a post office, two pubs, three banks and the sandwich shop.

University of York campus map (main campus)

Buses for the Banquet and Walking Tour will depart from here. This is also taxi pickup/drop off point

Accommodation

NSLC (**main conference venue**). Red dots show how to get to NSLC from the bus stop.

Alcuin reception – collect your accommodation keys

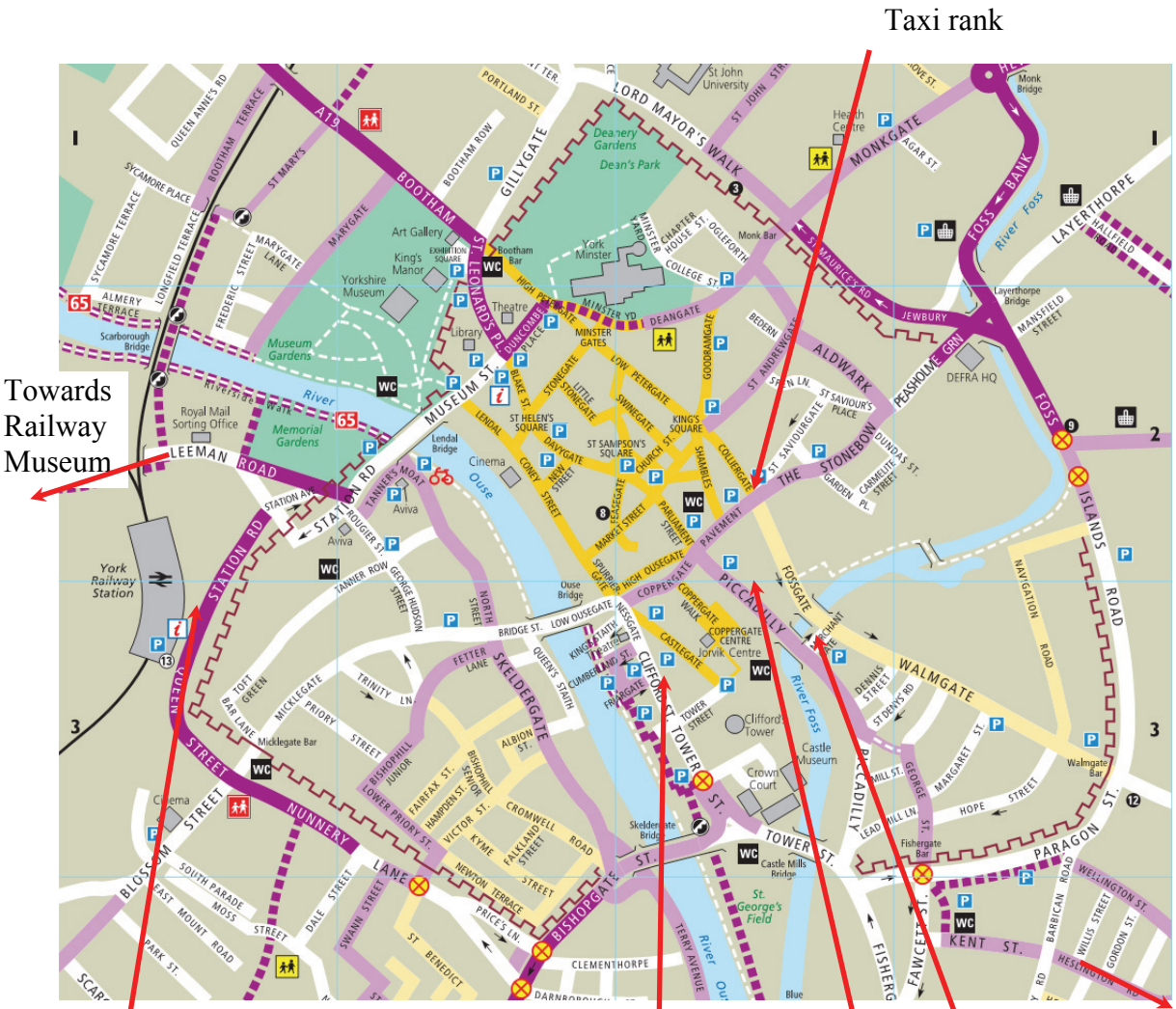
Derwent dining hall for lunches and dinners (except dinner on Sunday which is at NSLC)

Bus stop going to city (No. 4 or No. 44)

Bus stop coming from city (No. 4 or No. 44). Red dots show how to get to NSLC from the bus stop.

Car park and taxi pick up/drop off point

York city centre map



Speaker information

All lectures will be held in Lecture Theatre 1 in NSLC. A PC with PowerPoint and a projector will be provided. It will also be possible to attach a speaker's laptop to the projector. Laser pointer will be provided. If you need any other equipment, please inform conference organiser. **Please upload your presentation/test your laptop the day before your talk if at all possible.**

The length of lectures is 60, 30, 20 and 15 min for Bruker, Keynote, Invited, and all other lectures, respectively. Additional 5 min are allocated at the end of each lecture (except Bruker lecture) for questions.

Poster presenter information

Posters will be displayed in NSLC Atrium. Poster boards are **A0 portrait format**. They can be set up on Sunday afternoon, and will have to be taken down on Thursday before or shortly after lunch. Velcro adhesive tabs will be provided to attach the posters to the boards.

Poster numbers will be displayed on the boards. There will be two poster sessions at the conference, for even and odd posters. However, the posters will be on display throughout the conference, and coffee breaks/receptions will be held near the posters.

Poster prizes are sponsored by the British Biophysical Society and RSC.

Internet access

Delegates wishing to connect to the internet must bring their own laptops. In the main conference venue (lecture theatre, Atrium, bar), there are wireless networks EDUROAM and GUESTNET. You may be able to connect to EDUROAM using the username and password of your home institution (if they subscribe to EDUROAM). Alternatively, to get access to GUESTNET, you need to text the word "GUESTNET" to +44 1904 500205. The username and password will be texted back to you.

Delegates staying in single rooms: access to internet in the bedrooms can be purchased for £10 (valid for the duration of the conference). The delegates should bring their own CAT5 cable.

Delegates staying in double rooms: internet access in the rooms is free of charge using the GUESTNET username and password. Internet cable will be provided.

Car parking

Delegates bringing cars on campus will need a parking permit which should be requested from conference organiser in advance. The delegates with the parking permit should use the "Campus North" car park (nearest to the main conference venue, see map above).

Taxis

Two taxi points on campus (near accommodation and the main conference venue) are shown on the campus map above. Two taxi ranks in the city (at the

railway station and Colliergate) are shown on the city centre map. Conference organisers or porters will be happy to book taxis for the delegate, otherwise you may call Ebor cars at 01904-641441 or Streamline taxis at 01904-638833.

Checking out and left luggage

The delegates will need to check out **by 9.30 on the day of departure** (Thursday). It will be possible to store the luggage in a room at NSLC on Thursday until the end of the conference.

Accompanying persons

The registration fee for accompanying persons includes all lunches/dinners/banquet/reception. The only organised sightseeing at the conference will be the walking tour around York on Tuesday afternoon (please let organisers know in advance if you would like to take part). However, York has much more to offer!

Information centres are located at the railway station and in Museum Street (shown with red "*i*" icon on the city centre map above).

York Minster (the second largest Gothic cathedral in northern Europe) is shown on the city centre map. Near the Minster, visitors will find Treasurer's House and St William's College. York also has many fine churches open for viewing.

Museums: Castle museum, Yorkshire museum, National Railway museum, Clifford's tower, Jorvik centre (Viking village) are all shown on the city centre map. Other attractions include York Dungeon, Barley Hall, Merchant Adventurers Hall (where banquet is taking place).

Walking: In good weather, a walk on the city walls will give a splendid view of York (there are two small museums in Monk Bar and Monkgate Bar). There are many walking tours (including ghost tours, snickelways - narrow pedestrian routes).

Medieval streets, food/shops: The Shambles is the most famous medieval street in York (Britain's Most Picturesque Street in 2010) but the orange section of the map is the historic city centre with many tourist shops, restaurants/bars/café's and attractions.

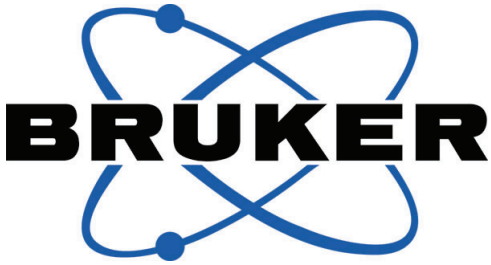
Theatres: York Theatre Royal and Grand Opera House.

Free afternoon and walking tour around York – Tuesday 5th April

The buses will take the delegates to the city centre (buses will leave at 14.00 from the Alcuin reception, see campus map above). There will be a 1h long walking tour starting from the Clifford's tower at 14.30 (please let the organisers know in advance if you want to take part). See the above section for accompanying persons for information about York attractions.

Delegates will have to make their own way back to campus for dinner. The best way is to catch a taxi or a bus No. 4 or No. 44 to the Morrell Library at the University of York (bus stops and taxi ranks are shown on the city centre map above).

Conference Sponsors



RSC | Advancing the Chemical Sciences

THE UNIVERSITY of York

EPR @ York

The EPR has been in the Chemistry Department at York since its foundation. Dick Norman, the founding head of Chemistry Department, and Bruce Gilbert (appointed a Lecturer in 1965), pioneered EPR research in York. Their interests focussed on the mechanistic chemistry of organic radicals. Dick and Bruce developed a flow system which was heavily used in conjunction with Fenton-type reactions to generate a variety of organic radicals in the EPR cavity and monitor their reactions. This method proved very successful and is still in our arsenal today. They also developed kinetic methods which made it possible to determine the rates of radical reactions from EPR data. Many experiments were carried out with spin traps (radical reactions with biomacromolecules were of particular interest) and new spin traps based on aci-nitroalkanes were developed for the flow systems.

One of the PhD students of Dick and Bruce, Mike Davies, was appointed an academic at York in 1990s. His research interests were in the radical reactions in biological systems. Mike then moved to Australia.

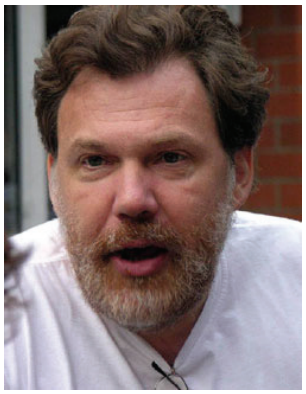
Victor Chechik was appointed at York in 1999. He had no prior exposure to EPR and was introduced to the technique in 2002 by Agneta Caragheorgheopol, a visitor in Bruce Gilbert's group. Victor then started using EPR in many of his projects. His interests cover spin labelling in supramolecular chemistry and nanoscience, mechanisms of free radical reactions, free radical reactions in biological systems. We currently have three cw-EPR instruments (Bruker ESP-300E, JEOL JES-RE1X and Bruker EMX micro).

The York people have always supported the ESR group of the RSC. Dick and Bruce were at the meeting in Cardiff in 1968 which founded the ESR group of the RSC. Bruce was a Chair of the group. Adrian Whitwood (who did his PhD in Bruce's group and then for many years supported EPR research in the Department) was a committee member of the ESR group for several years. Victor was a Secretary and a Treasurer of the group. York hosted many conferences of the ESR group of the RSC, starting from 1971, and then 1975, 1980, 1987, 1992, 1999 and 2011.

Bruker prize lecture and reception

Since 1986 Bruker BioSpin have generously sponsored an annual lectureship and prize, given to a scientist who has made major contributions to the application of ESR spectroscopy in chemical or biological systems.

The Bruker Lectureship for 2011 has been awarded to:



Thomas Prisner

University of Frankfurt
Germany

The lecture will take place on Tuesday 5th April in the Lecture Theatre 1 (NSLC) at 19.30, followed by the Bruker-sponsored Wine Reception in the NSLC Atrium and a Free Bar also kindly sponsored by Bruker.

The title of Bruker lecture 2011 will be:

New developments in EPR and DNP and application to biomolecular research

Previous winners of the Bruker Lectureship:

1986	M. C. R. Symons	**1995**	H. M. McConnell	**2004**	W. L. Hubbell
1987	K. Möbius	**1996**	B. M. Hoffman	**2005**	K.-P. Dinse
1988	H. Fischer	**1997**	K. A. McLauchlan	**2006**	Yu. D. Tsvetkov
1989	J. S. Hyde	**1998**	J. R. Pilbrow	**2007**	D. Goldfarb
1990	J. H. Freed	**1999**	J. Schmidt	**2008**	E. J. J. Groenen
1991	E. de Boer	**2000**	D. Gatteschi	**2009**	G. Jeschke
1992	G. Feher	**2001**	J. Hütterman	**2010**	R. P. Mason
1993	N. M. Atherton	**2002**	G. R. & S. S. Eaton		
1994	A. Schweiger	**2003**	W. Lubitz		

JEOL student prize lectures

The JEOL competition is open to postgraduates in their 2nd or 3rd year and postdoctoral fellows in their 1st year. The 15 minutes lectures are judged by the ESR Spectroscopy Group Committee on the basis of their scientific content and delivery. An engraved medal and monetary prize are generously provided by JEOL for the winner of the presentation, to be presented at the conference banquet.

This year, the competition will take place during the Monday afternoon session. The 2011 lectures, selected on the basis of the abstracts submitted, will be:

In-cell PELDOR on Nucleic Acids

Ivan Krstić

Institute of Physical and Theoretical Chemistry, Goethe University Frankfurt

Distance Measurements on Lanthanide Ion – Nitroxide Radical Spin Pairs in the Nanometre Range by Relaxation Enhancement

Petra Lüders

Laboratory of Physical Chemistry, ETH Zurich, Switzerland

A Single Supernumerary Cysteine Adjacent to the Proximal Fe-S Cluster Confers Oxygen Tolerance on a [NiFe]-Hydrogenase

Maxie Roessler

Department of Chemistry, University of Oxford, U.K.

The wine reception in NSLC Atrium on Monday evening is kindly sponsored by JEOL, followed by a Free Bar, also kindly sponsored by JEOL.

Committee of the ESR Spectroscopy Group of the Royal Society of Chemistry

Dr Mark Newton (Chair)	University of Warwick	2010-2013
Dr Chris Kay (Secretary)	University College London	2006-2011
Dr Fraser MacMillan (Treasurer)	University of East Anglia	2010-2014
Dr Ilya Kuprov (Web Master)	University of Oxford	2009-2012
Dr Rachel Haywood	RAFT Institute	2008-2011
Dr Rob Hill (Industry Representative)	Bruker	2008-2011
Dr Damien Murphy	University of Cardiff	2008-2011
Dr David Norman	University of Dundee	2009-2012
Dr Christiane Timmel	University of Oxford	2009-2012
Dr Victor Chechik	University of York	2010-2011
Prof David Collison	University of Manchester	2010-2011
Dr Graham Smith	University of St Andrews	2010-2013
Dr Dima Svistunenko	University of Essex	2010-2013
Dr Helen Williams	AstraZeneca	2010-2013

Victor Chechik would like to acknowledge the help of people at York who assisted with the conference organisation:

Prof Bruce Gilbert
Dr Adrian Whitwood
Dr Jamie Gould
Thomas Newby
Kazim Naqvi
Rob Smith
Chiara Baldassarri
Zhou Lu

2012

45th Annual International Meeting of the ESR Spectroscopy Group of the Royal Society of Chemistry

Sunday 25 to Thursday 29 March 2012

Using spin traps in heterogeneous reactions

Victor Chechik

Department of Chemistry, University of York, Heslington, York YO10 5DD, UK

EPR spectroscopy of spin adducts is one of the most direct methods for detecting short-lived free radical intermediates. While most applications of spin trapping remain in the biological environment, this method can be successfully used for probing chemical reactions in other systems.

In particular, heterogeneous catalysis is an area where organic radicals are playing an important role. Rich redox chemistry of transition metals can lead to generation of either free or adsorbed radicals. However, obtaining mechanistic information in heterogeneous systems is very difficult. We believe therefore that spin trapping has much to offer in this area.

We have used spin trapping to explore reactivity of Au nanoparticles. Despite being very inert in the bulk form, nanoparticulate gold is quite reactive, particularly in oxidation reactions. Some of these reactions proceed via radical intermediates. It is difficult to establish the precise factors that govern the nanoparticle reactivity but the nature of the adsorbed ligand seems to play an important role. Our most recent project is aimed at deciphering whether the reactivity of Au nanoparticles in oxidation reactions is linked to their behaviour in biological systems.

We will also discuss detecting free radicals in heterogeneously catalysed reactions in the gas phase. We have built a reactor which traps the gas phase radicals shortly after they leave the catalytic bed. The reactor has been successfully used to explore the gas-phase oxidation reactions.

References.
[1] B. Mile*, Curr. Org. Chem.,* **2000,** *4,* 55.
[2] M. Conte, H. Miyamura, S. Kobayashi, V. Chechik, *J. Am. Chem. Soc.* **2009,** *131*, 7189.
[3] M. Conte, V. Chechik, *Chem. Commun.,* **2010,** *46,* 3991.

Combined ST/EPR and LC-MS approach to identify spin adducts and investigate the reactivity of beer bitter acids towards 1-hydroxyethyl radical

Natália Ellen Castilho de Almeida*, Daniel Rodrigues Cardoso*
*Institute of Chemistry at São Carlos, University of São Paulo, Brazil.

Electron paramagnetic resonance (EPR) spin-trapping (ST) technique have demonstrated that 1-hydroxyethyl (HER) radical is the main radical species produced during the beer aging process [1]. Beer is an alcoholic beverage made from water, barley malt, hops (*Humulus lupulus)* and yeast. Indeed, hop-derived compounds are essential for the typical bitter taste and flavor of beer and for the bacteriostatic activity during wort boiling [2]. However, iso-α-acids and beta-acids are very sensitive to oxidative decomposition and most oxidation reaction products possess unpleasant organoleptic characteristics [3]. The present work describes the reactivity of iso-α-acids and β-acids towards HER as probed by EPR/ST technique-using 4-POBN (spintrap) in conjunction with LC-MS to identify spin adducts and oxidation products. The apparent second-order rate constants were obtained using a competitive kinetics approach. It was observed that both diastereoisomers *cis*- and *trans*-iso-α-acids are degraded in the presence of HER with k_{app} = 1.8 10^8 and k_{app} = 9.2 10^9 L mol^{-1} s^{-1}, respectively. The reactivity of dihydro-iso-α-acids towards HER was similar to the reactivity of the iso-α-acids diastereoisomeric mixture (k_{app} = 1.5 10^9 L mol^{-1} s^{-1}). On the other hand, the tetrahydro-iso-α-acids analogues did not reacted with HER. The β-acids show similar reactivity of the *cis*-iso-α-acids, k_{app} = 2.6 10^8 L mol^{-1} s^{-1}. The detected spin adducts by combined EPR/ST and LC-MS were 4-POBN/HER spin adduct, it's oxidized form and the species formed by binding 4-POBN with two molecules of HER. It was observed an increase of the latter two species, as function of the reaction time, which thereby may lead to underestimation of the apparent rate constants for ST experiments employing longer incubation times. The reactive sites could then be assigned to the allylic positions in the side chains and hydrogen abstractions lead to stabilized allylic radicals that eventually are trapped by radicals. The final reaction products are hydroxyl-alloisohumulones and positional isomers of hydroxylated isohumulones. The results of this study are of great importance for understanding essential processes involved in beer aging and, furthermore, the developed competitive kinetic approach is of importance for further studies involving the 1-hydroxyethyl radical in food or biological systems.

This research is supported by FAPESP (Proc. 2009/00858-0).

[1] Andersen, M.L.;Skibsted, L.H. *J. Agric. Food Chem.* **1998**, *46*, 1272-1275.
[2] Van Cleemput, M. et al. *J. Nat. Prod.* **2009**, *72*, 1220-1230.
[3] Haseleu, G. et al. *J. Agric. Food Chem.* **2010**, *58*, 7930-7939.

Synthesis of different 5-alkoxycarbonyl-4-hydroxymethyl-5-methylpyrroline *N*-oxide derivatives

A. Patel[1], K. Stolze[2], N. Rohr-Udilova[3], and T. Rosenau[1].

[1]Dept. Chemistry, University of Natural Resources and Life Sciences (BOKU), Muthgasse 18, A-1190 Vienna, Austria
[2]Institute of Pharmacology and Toxicology, Dept. Biomedical Sciences, University of Veterinary Medicine Vienna, Veterinärplatz 1, A-1210 Vienna, Austria.
[3]Div. Gastroenterology and Hepatology, Clinic of Internal Medicine III, Medical University of Vienna, A-1090 Vienna, Austria

Synthesis and free radical trapping properties of a series of spin traps derived from the spin trap 5-ethoxycarbonyl-5-methyl-pyrroline *N*-oxide (EMPO) are reported, which is a derivative of the wellknown spin trap 5,5-dimethyl-pyrroline *N*-oxide (DMPO), but has improved spin trapping properties towards oxygen-centered radicals, especially towards superoxide radicals, one of the most important reactive oxygen species (ROS) in biological systems.
Our goal was to introduce reactive functional groups to which site-selective moieties can be attached according to the specific needs of the biological system to be tested. In this respect we synthetized EMPO derivatives with an additional 4-hydroxymethyl side chain, namely 5-ethoxycarbonyl-4-hydroxymethyl-5-methylpyrroline *N*-oxide (EHMPO), 5-ethoxycarbonyl-5-ethyl-4-hydroxymethylpyrroline *N*-oxide (EEHPO), 4-hydroxymethyl-5-methyl-5-propoxycarbonylpyrroline *N*-oxide (HMPPO), and 4-hydroxymethyl-5-methyl-5-*iso*-propoxycarbonylpyrroline *N*-oxide (HMiPPO). All compounds were characterized by ^{1}H and ^{13}C NMR. The spin trapping activity of these compounds towards different carbon-centered radicals derived from methanol, ethanol, and formic acid, generated in the presence of a Fenton-type system, was also tested.

This research is supported by the Christian-Doppler Research Society (CD lab "Advanced cellulose chemistry and analytics".

An ESR method to determine relative volatility of HALS in surface coatings.

Philip J Barker[*], Martin R L Paine[**], Stephen J Blanksby[**]

[*]BlueScope Steel Research, P O Box 202, Port Kembla, NSW 2505, Australia
[**]School of Chemistry, University of Wollongong, NSW 2522, Australia

The durability of surface coatings of many types, in many applications may be enhanced by the use of hindered amine light stabilisers (HALS), which interfere with radical mediated oxidative events that would otherwise lead to coating degradation. The enhancement depends upon many factors, but at BlueScope Steel our flagship product for domestic roofing, guaranteed for 25 years (under harsh Australian exposure conditions), employs a paint system formulated with a commercial HALS molecule incorporating two piperidinyl functionalities, to assist in maintaining aesthetic performance in the first 5-10y of service life.

When one of our 150m/min coil coating lines experienced a operational problem in the filter room of a High Velocity Air Cooler, which cools the painted steel strip by 20ºC after curing, prior to water quenching, the problem was tracked to sticky residues from a poorly formulated backing coat. These residues blocked the filters, causing unnecessary and expensive line stoppages. During the analysis phase of the investigation, HALS was found to be overrepresented in the residues. ESR measurements indicated elevated levels of HALS monoradical in the residue and elevated HALS biradical levels after p-nitroperbenzoic acid oxidation [1]. A simple semi-quantitative ESR analysis indicated that up to 15% of the original concentration of HALS could be lost in processing, in turn representing a significant financial loss across 5 coating lines using over 6 million litres of HALS-containing topcoat paint and producing, in total, over 500,000 tonnes of pre-painted steel product per year.

To ensure that both current and alternative coatings (including primers topcoats and backers) would not cause a similar problem, a static experimental rig was designed to mimic the coil coating process, collecting volatile material in a cold-trap. This rig could then be used to evaluate, after a further simple ESR analysis, the relative volatilities of a range of commercial HALS molecules formulated in a common paint system.

The results are of significance to all coatings and polymer sectors where HALS are employed and concur with recent studies upon HALS activation under coil coating processing conditions [2].

1. J L Gerlock, A V Kucherov, C A Smith Polymer *Degradation and Stability* 2001, ***73,*** 201.
2. M R L Paine, P J Barker, S J Blanksby *Analyst* 2011, ***136***, 904.

Non-covalent and site-directed spin labeling of nucleic acids

Snorri Th. Sigurdsson

Science Institute, University of Iceland, Dunhaga 3, 107 Reykjavik, Iceland.

Studies of nucleic acids by Electron Paramagnetic Resonance (EPR) spectroscopy require spin-labeled oligomers. Several different site-directed spin labeling (SDSL) methods have been developed for nucleic acids over the years for incorporation of nitroxides at specific sites. These methods have utilized either of two approaches, incorporation of the spin label during chemical synthesis of the nucleic acid or post-synthetic modification with a spin-labeling reagent. Both of these strategies require incorporation of the spin label through chemical reactions. Some of the potential drawbacks associated with these chemical modifications include a labor-intensive preparation of the spin label, incomplete labeling, side-reactions and purification of the labeled oligomer.

To avoid labor-intensive and costly chemical modifications of the biopolymer, we have developed a non-covalent and site-directed spin labeling (NC-SDSL) approach for nucleic acids.[1] The strategy utilizes a spin label that binds site-specifically and non-covalently to abasic sites in nucleic acids. Thus, spin-labeled samples can be prepared by simply mixing the spin-labeling reagent with a solution of the nucleic acid, prepared from commercially available phosphoramidites.

This research was supported by the Icelandic Research Fund and the University of Iceland Research Fund.

[1] Shelke SA, Sigurdsson ST, “Non-covalent and site-directed spin-labeling of nucleic acids,” *Angew. Chem. Int. Ed.* **2010**, *49*, 7984-6.

Characterization of supramolecular systems by EPR spectroscopy.

Elisabetta Mileo[*], Angel E. Kaifer[**], Marco Lucarini[***]

[*]Laboratoire Chimie Provence, Université de Provence, Marseille.
[**]Centre of Supramolecular Chemistry, University of Miami, FL.
[***]Dipartimento di Chimica Organica "A. Mangini", Università di Bologna.

Molecules that can recognize other molecules or ions, mixtures of molecules that can self-assemble into racks, rosettes or ribbons, molecular machines, and molecules that can mimic life by self-replicating may sound like science fiction, but are actually examples of the progress made in the area of *supramolecular chemistry* in the last 30 years. Familiar molecules such as methane (CH_4) and carbon dioxide (CO_2) are made up of atoms joined together by strong chemical interactions called covalent bonds. Molecular chemistry is concerned with making and breaking covalent bonds to form new molecules. Supramolecular chemistry *is different* because it deals with synthetic molecular systems that are held together by weaker non-covalent interactions, such as electrostatic forces, hydrogen bonds, π-π stacking interactions, van der Waals forces, or hydrophobic effects. These non-covalent interactions taken individually are weak, but when several are used together, very stable molecular ensembles or complexes may result [1].

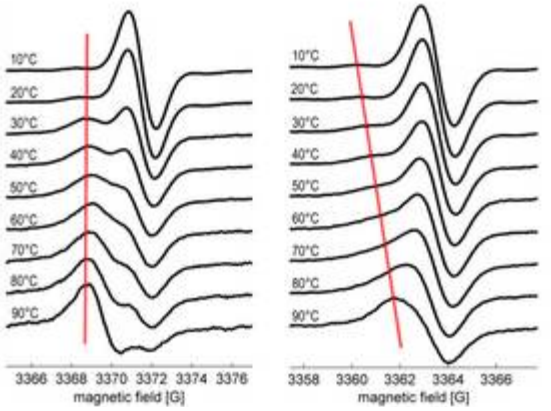
In this work we show how the peculiar features of nitroxide radicals to be sensitive to their environment, with a subsequent change of the spectroscopic parameters upon complexation or self-assembly, can be useful to study several supramolecular entities such as cucurbiturils complex [2], resorcinarene molecular capsules [3] and pseudorotaxanes [4].

[1] J. M. Lehn *Supramolecular Chemistry. Concepts and perspective*. **1995**, VHC, Weinheim, Germany.
[2] E. Mileo, E. Mezzina, F. Grepioni, G. F. Pedulli, M. Lucarini *Chem. Eur. J.* **2009**, *15*(32), 7895-7862.
[3] E.Mileo, S. Yi, P. Bhattacharya, A.E. Kaifer. *Angew. Chem .Int. Ed.* **2009**, *48* (29), 5337- 5330.
[4] E. Mileo, C. Casati, P. Franchi, E. Mezzina, M. Lucarini *Org. Biomol. Chem.* **2011**, Accepted Manuscript.

Spin-Probing Nanoscopic Inhomogenities in Pluronics

Dennis Kurzbach*, Miriam Reh*, Dariush Hinderberger *
*Max Planck Institute for Polymer Research, Ackermannweg 10, 55128 Mainz.
Corresponding contact: kurzbach@mpip-mainz.mpg.de

In this report we present continuous wave (cw) electron spin resonance (ESR) spectroscopy data gained from spin probing PEO-PPO-PEO tri-block copolymers (Pluronic® or Poloxamer) with 2,2,6,6-tetramethylpiperidine-1oxyl (TEMPO). Notably smaller than the conventionally in the context of polymers used spin probes, TEMPO reveals the emergence of smallest hydrophobic cavities on the onset of a collapse of the PPO strand of several Pluronics (varying overall molecular weight and the PEO/PPO ratio) when the temperature is increased. The occurrence of hydrophobic cavities (indicated by smaller a_{iso} and g_{iso} values of the spin probes) seems to be mainly independent of the overall molecular weight of the Pluronics under investigation, but depends clearly on the PPO/PEO ratio, although published data indicates higher molecular weight to be responsible for faster collapse of the PPO blocks [1]. The temperature range in which the amount of hydrophobic cavities increases is ≥ 40°C (while bigger spin probes lead to values of about 10-20°C, in which micellization can be observed [2]) in any case observed and is not correlated directly to micellization of the polymers. A decrease of the relative PPO amount in the polymers not only hinders collapse of PPO strands, it also leads to an exchange of the spin probes between hydrophobic cavities and the surrounding medium, while in cases of high PPO content spin probe exchange could not be observed (Figure above). As observed separately in two other classes of thermoresponsive materials, in Pluronics, nano-scale inhomogeneities can be observed that are static [3] (high PPO content) and dynamic [4] (low PPO content) on the ESR timescale. Exchange of guest molecules between the hydrophobic cavities formed by PPO strands and the surrounding medium has not been observed so far by ESR techniques and therefore most likely depends on the size and hydrophobicity of the spin probe. Such exchange phenomena could play a crucial role, when applications of Pluronics as drug carriers are concerned.



CW spectra of the I_N = -1 transition line of TEMPO in a P105 (left) and F108 (right) solution. Red lines indicate transitions of the encapsulated species.

1. Wanka, G., H. Hoffmann, and W. Ulbricht, Macromolecules, 1994. **27**: p. 4145-4159.
2. Caragheorgheopol, A., et al., Langmuir, 1997. **13**: p. 6912-6921.
3. Junk, M.J.N., U. Jonas, and D. Hinderberger, Small, 2008. **4**: p. 1485-1493.
4. Junk, M.J.N., et al., Angew. Chem. Int. Ed., 2010. **49**: p. 5683-5687.

An electrochemical and EPR spectroelectrochemical study of *peri*-substituted naphthalenes

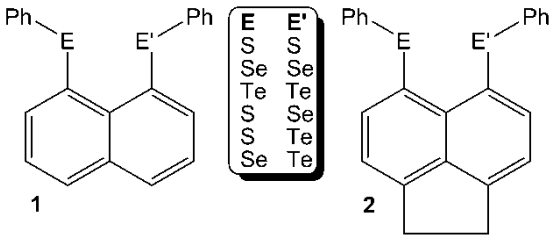
R.T. Boeré[*], T.L. Roemmele[*], L.K. Aschenbach[**], R.A.M. Randall[**], F.R. Knight[**], M. Bühl[**], A.M.Z. Slawin[**], J. D. Woollins[**]

[*]Dept of Chemistry and Biochemistry, University of Lethbridge, Lethbridge, AB Canada, T1K 3M4 (Fax: +1-403-329-2057, Email: boere@uleth.ca)
[**]School of Chemistry, University of St Andrews, St Andrews, Fife, KY16 9ST (UK) (Fax: +44-1334-463384 Email: jdw2@st-and.ac.uk)

A systematic series of naphthalenes and acenaphthenes *peri*-substituted by PhE (E = S, Se, Te) has been synthesized and characterized by crystallography, spectroscopy and DFT computational studies [1]. *peri*-Substitution is a double substitution in positions 1 and 8 of the naphthalene ring. The distance between the chalcogen atoms due to *peri*-substitution is less than the sums of van der Waals' radii of the atoms and the bonding environment is currently a subject of intensive discussion [2].

Based on electron-counting and simple electronic-structure considerations, *removal* of valence electrons is predicted to increase the degree of attractive interaction between the chalcogen atoms. In order to assess the validity of this hypothesis and gain insight on suitable reagents for the synthesis and isolation of suitably oxidized species, a detailed voltammetric investigation has been undertaken.

Here we report on the voltammetric behaviour of six 1,8-$(PhE)_2$-$C_{10}H_6$ *peri*-naphthalenes (E = S,Se,Te) **1** and the related series of acenaphthenes **2**. The redox-potentials are strongly dependent on the identity of E and E', and the products of one-electron oxidation have been identified by EPR spectroelectrochemistry. Interpretation of the data was assisted by DFT calculations on starting materials and oxidized species.



The novel application of *in situ* EPR methods to confirm voltammetric processes for redox-active compounds of the main group elements will be specially highlighted.

[1] F.R. Knight, A.L. Fuller, M. Bühl, A.M.Z. Slawin and J.D. Woollins, *Chem. Eur. J.* **2010** *16*, 7503.
[2] P. Kilian, F.R. Knight and J.D. Woollins, *Chem. Eur. J.* **2011** *17*, 2302.

In-cell PELDOR on Nucleic Acids

Ivan Krstić[*], Robert Hänsel[**], Olga Romainczyk[***], Joachim W. Engels[***], Volker Dötsch[**], Thomas F. Prisner[*]

[*] Institute of Physical and Theoretical Chemistry, Goethe University Frankfurt.
[**] Institute of Biophysical Chemistry, Goethe University Frankfurt.
[***] Institute of Organic Chemistry and Chemical Biology, Goethe University Frankfurt.

Tertiary structure and conformational dynamics of RNA are essential for its function as a biological catalyst, regulator or structural scaffold [1]. Over the past few years pulsed electron-electron double resonance (PELDOR) [2] spectroscopy has demonstrated its applicability to determine distances in nucleic acid (NA) molecules in the range of 1.5–6 nm.

NA structure and dynamics depend on environmental factors, such as concentration of ions, small molecules, molecular crowding, viscosity and interactions with proteins. Therefore it is important to investigate if the *in vitro* determined NA structure reflects the intracellular (*in vivo*) conformation. In the present study we used PELDOR spectroscopy on a double-labeled 12-base pair DNA duplex, the 14-mer cUUCGg tetraloop hairpin RNA and the 27-mer neomycin-sensing riboswitch [3] to obtain long-range distance constraints on such systems in *Xenopus laevis* oocytes and to compare them with *in vitro* measurements.

The reduced lifetime of nitroxide radicals under *in vivo* conditions is a major obstacle for such measurements. The 5-membered pyrrolidine and pyrroline rings get significantly slower reduced compared to 6-membered piperidine ring based nitroxides. Our results show that in comparison to free nitroxide the in-cell reduction kinetic for the spin label attached to NAs is much slower.

We report the first application of PELDOR spectroscopy to map the global structure of nucleic acids inside *Xenopus laevis* oocytes. No alterations in the measured distances between *in vitro* and in-cell experiments implies the existence of stable overall conformations of the 14-mer hairpin RNA and the 27-mer neomycin-sensing riboswitch, whereas the 12-bp duplex DNA experiences stacking in-cell but retaining the secondary structure.

These results lay a foundation for the application of PELDOR spectroscopy for probing structural aspects of NAs through the cell-cycle progression.

[1] R. F. Gesteland, T. Cech, J. F. Atkins, *The RNA world*, 3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., **2006**.
[2] a) A. D. Milov, A. B. Ponomarev, Y. D. Tsvetkov, *Chem. Phys. Lett.* **1984**, *110*, 67; b) O. Schiemann, T. F. Prisner, *Q. Rev. Biophys.* **2007**, *40*, 1; c) G. Jeschke, Y. Polyhach, *Phys. Chem. Chem. Phys.* **2007**, *9*, 1895.
[3] I. Krstić, O. Frolow, D. Sezer, B. Endeward, J. E. Weigand, B. Suess, J. W. Engels, T. F. Prisner, *J. Am. Chem. Soc.* **2010**, *132*, 1454.

Distance Measurements on Lanthanide Ion – Nitroxide Radical Spin Pairs in the Nanometre Range by Relaxation Enhancement

Petra Lueders, Maxim Yulikov, Gunnar Jeschke

Laboratory of Physical Chemistry, ETH Zurich, Switzerland.

The relaxation enhancement of a nitroxide spin probe, induced by a fast relaxing paramagnetic centre, can potentially be used as an alternative to DEER experiments to measure distances in the nanometre range [1].
In the present work we explore the performance and reliability of relaxation enhancement based measurements for distance determinations. The best results are obtained from the T_1 relaxation enhancement in Dy^{3+} - nitroxide spin pairs.
Multiple frequency studies (S, X, and Q band) show an increase of relaxation enhancement and therefore an increase of the addressable distance range from Q- to S-band frequencies.
The presence of oxygen in the lipid bilayer causes non-additive relaxation enhancement and leads to an underestimation of the Dy^{3+} - nitroxide distance.
Comparison to DEER shows that the mean distances can be obtained with a moderate precision of ~ 20 %. We also discuss the effect of averaging with a r^{-6} power law on the apparent mean distance.
The magnitude and scaling of relaxation enhancement measured in labelled model polypeptides incorporated into lipid bilayers suggest the possibility to measure distances up to ~ 6 nm at X band.

[1] H. Jaeger, A. Koch, V. Maus, H.W. Spiess, G. Jeschke, Journal of Magnetic Resonance 194 (2008) 254

A Single Supernumerary Cysteine Adjacent to the Proximal Fe-S Cluster Confers Oxygen Tolerance on a [NiFe]-Hydrogenase

Maxie M. Roessler[ab], Michael J. Lukey[a], Rosalind Davies[a], Alison Parkin[a], Rhiannon Evans[a], Jeffrey Harmer[b], Fraser Armstrong[a]

[a]*Department of Chemistry and* [b]*Centre for Advanced Electron Spin Resonance (CAESR), University of Oxford, South Parks Road, Oxford OX1 3QR, U.K.*

The intriguing manner by which certain [NiFe]-hydrogenases survive attack by O_2 is a topic of considerable importance because these enzymes, or inspired synthetic catalysts, hold great promise for future H_2-energy technologies.[1] The [NiFe]-hydrogenases consist minimally of a large subunit, which houses the active site (Ni and Fe with thiolates, CO and CN ligands), and a small subunit containing at least one electron-transferring iron-sulfur cluster. In respiratory [NiFe]-hydrogenases, three Fe-S clusters proximal, medial, and distal to the active site form a relay to the surface of the protein.[2] Standard [NiFe]-hydrogenases, which operate under anaerobic conditions *in vivo*, are inactivated by traces of O_2.[2] Oxygen attack results, at best, in the so-called 'Unready' state (Ni_u-A) that is re-activated very slowly upon reduction.[3] However, some [NiFe]-hydrogenases, such as Hydrogenase-1 (Hyd-1) from *Escherichia coli*, sustain high activity under aerobic conditions and thus are defined as 'O_2 tolerant'.[4]

Oxygen tolerance has been linked to the following observations: (1) avoidance of the formation of the Ni_u-A state; (2) an unusual EPR spectrum in which the signal of the $[3Fe\text{-}4S]^+$ medial cluster shows 'wings' attributed to spin coupling with another paramagnetic site, suggesting that a further electron can be released by the Fe-S relay under mild oxidizing conditions; (3) two conserved cysteine residues in O_2-tolerant respiratory hydrogenases (C19 and C120 in the small subunit HyaA of Hyd-1) that correspond to glycines adjacent to the [4Fe-4S] proximal cluster in standard hydrogenases. We have therefore systematically exchanged C19 and C120 in Hyd-1 to glycine, with the key aim to establish if *either* or *both* cysteines have evolved to promote O_2 tolerance and simultaneously, are responsible for the complex EPR signal observed at high potential.

Native Hyd-1, and three variants Hyd-1 HyaA C19G/C120G, C19G, and C120G are compared using the complementary techniques of EPR spectroscopy and protein film electrochemistry. Our findings are discussed with regard to observations (1) and (2) above, and we conclude that C19 is the crucial residue. The ability of a respiratory [NiFe]-hydrogenase (Hyd-1 from *E. coli*) to sustain activity in the presence of O_2 thus depends critically upon a special feature of the small subunit – a supernumerary cysteine (C19) located next to the proximal iron-sulfur cluster.

This research is supported by EPSRC (Grant EP/D044855D/1 supporting the CAESR) and BBSRC (Grant H003878-1).

[1] Cammack, R.; Frey, M.; Robson, R. *Taylor and Francis: London and New York* **2001**.
[2] Fontecilla-Camps, J. C.; Volbeda, A.; Cavazza, C.; Nicolet, Y. *Chem Rev* **2007**, *107*, 4273.
[3] Lamle, S. E.; Albracht, S. P.; Armstrong, F. A. *J Am Chem Soc* **2005**, *127*, 6595.
[4] Lukey, M. J.; Parkin, A.; Roessler, M. M.; Murphy, B. J.; Harmer, J.; Palmer, T.; Sargent, F.; Armstrong, F. A. *J Biol Chem* **2010**, *285*, 3928.

PROTEIN CONFORMATIONAL DYNAMICS FROM SPIN LABELING EPR SPECTROSCOPY

Hassane S. Mchaourab

Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN 37232

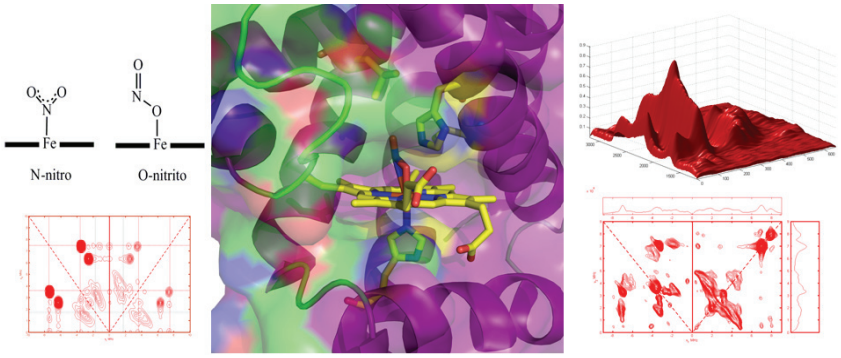
My laboratory uses spin labeling and EPR spectroscopy to define conformational rearrangements underlying the function of a broad spectrum of proteins. Specific examples include alternating access in multidrug- and neurotransmitter transporters which exposes the substrate binding site to either side of the membrane and the response of calcium-calmodulin dependent protein kinase to calcium signals, its activation and the structural basis of conformational memory. The main EPR tool is by Double Electron-Electron Resonance (DEER) spectroscopy for long range distance measurements between spin label pairs. The average distances from DEER yield the amplitude of protein motion. Furthermore, analysis of the distance distribution can reveal equilibria between multiple conformational intermediates. Newly developed methodology to increase the DEER throughput and distance range in lipid bilayers will be illustrated. Specifically the synergistic convergence of two technologies, discoidal nanoscale lipoprotein-bound bilayers and Q-band (34 GHz microwave frequency) pulsed EPR, circumvent the factors limiting DEER sensitivity and distance range in lipid bilayers thereby effecting a significant increase in experimental throughput. These advances enable detailed insight into the mechanism of these proteins in a native-like environment in the absence of conformational selectivity imposed by the crystal lattice.

Myoglobin and nitrite: an EPR investigation of a physiologically relevant new role

Matt Bawn, Christopher Stephens, Fraser MacMillan

Henry Wellcome Unit for Biological EPR, School of Chemistry, University of East Anglia, Norwich, NR4 7TJ, UK.

The primary function of myoglobin (Mb) has long been considered to be the cellular storage and supply of oxygen [1]. Recently, however, a role has been suggested for Mb as a nitrite reductase [2], where the nitrite anion is reduced to nitric oxide ($NO^{\bullet}$). Under hypoxic conditions $NO^{\bullet}$ is able to regulate cardiac energy consumption and reduce myocardial oxygen consumption thereby reducing the risk of ischemic-reperfusion injury [3], a major source of tissue damage after periods of vasoconstriction as may be experienced after a stroke or heart attack.



To understand the mechanism of nitrite reduction by Mb it is necessary to examine the binding of the anion to the protein substrate, which is the iron centre of a haem moiety. Crystal structures of horse-heart Mb [4] and human haemoglobin (Hb) [5] (hypothesised to be functionally related to Mb), suggest that the anion adopts the uncommon *O*-nitrito binding mode, in which the haem iron is directly coordinated to an oxygen of nitrite.

In order to determine whether this binding mode occurs in protein unconstrained by crystallisation, electron paramagnetic resonance (EPR) spectroscopy has been employed. To better understand the behaviour of Mb under varying physiological conditions the protein was examined over a range of pH values [pH 5.0-10.8]. Continuous-wave (cw) EPR at S, X, & Q-band initially provide insights into the local haem environment, and are complimented by hyperfine spectroscopic techniques (ESEEM [6] & HYSCORE [7]) to resolve the mode of nitrite binding.

[1] B. A. Wittenberg *et al*, *Journal of Biological Chemistry* **1975**, *250*, 9038.
[2] T. Rassaf *et al Circ Res* **2007**, *100*, 1749.
[3] M. L. Hess *et al*, *Journal of Molecular and Cellular Cardiology* **1984**, *16*, 969.
[4] D. M. Copeland *et al*, *Journal of Inorganic Biochemistry* **2006**, *100*, 1413.
[5] J. Yi, *et al*, *Biochemistry* **2008**, *47*, 8247.
[6] Y. Deligiannakis *et al*, *Coordination Chemistry Reviews* **2000**, *204*, 1.
[7] P. Hofer *et al*, *Chemical Physics Letters* **1986**, 132, 279.

The catalatic mode of *Mycobacterium tuberculosis* catalase-peroxidase as studied at different pH values

Dimitri A. Svistunenko, Jonathan Worrall and Peter Nicholls

Department of Biological Sciences, University of Essex, Wivenhoe Park, Colchester, Essex CO4 3SQ, UK.

The bi-functional enzyme catalase-peroxidase (KatG) from *Mycobacterium tuberculosis* is responsible for turning the pro-drug INH into a chemically active form capable of killing the *M. tuberculosis* bacterium. We used UV-visible optical spectroscopy and low temperature X-band CW EPR spectroscopy to study optically active and paramagnetic forms of the enzyme both in the resting ferric state and in the intermediates formed under an excess of hydrogen peroxide. The effects of the pH on the static (resting state) ferric haem forms, on the kinetics of the intermediates and on the kinetic parameter of the catalatic activity were studied. The rapid freeze-quench method of making EPR samples was employed. For the first time, the concentrations of the enzyme for the EPR experiments were as low as those used in the optical spectroscopy. This has allowed us to obtain the kinetic dependences of some species by two different methods. The fact that those dependences for the ferric haem state were found to be 'superimposable' (the result we have been looking for before but were not able to obtain) points to the importance of sample preparation by the rapid freeze-quench method and opens up new promising opportunities to create computer kinetic models based on the experimental data obtained - in full consistency - by two different types of spectroscopy.

Dynamics and topology of spin-labeled lipids and peptide antibiotics in model phospholipids membranes probed by stimulated ESE

Sergei A. Dzuba

Institute of Chemical Kinetics and Combustion, Russian Academy of Sciences, 630090 Novosibirsk, Russia

Three-pulse stimulated electron spin echo (ESE) technique is sensitive to fast stochastic librations of spin labels, with correlation time in the nanosecond scale, and to their slow millisecond rotations, which in the accessible microsecond time scale manifest themselves as small-angle reorientations. Spin-labeled phospholipids with different position of the label were studied in POPC and DPPC bilayers. Both types of motion were found to persist even at cryogenic temperatures. Dynamics of lipids in POPC and DPPC bilayers were found to be remarkably different. It was found also that presence of cholesterol influences motions in essentially different way for different positions of the label. The results are discussed in the framework of the membrane dynamics and topology.

Also, spin-labeled analogs of the antimicrobial lipopeptide trichogin GA IV, in which TOAC spin label is rigidly attached to the peptide backbone, were studied in POPC membranes. At low molar peptide to lipid (P/L) ratio, only fast librational motions were detected. At high P/L ratio also slow rotations appear. The peptide location in the membrane was studied applying stimulated ESE envelope modulation (ESEEM) phenomenon for the D_2O-hydrated lipids. It was found that at low P/L ratio individual peptide molecules are randomly distributed at the membrane surface while at high P/L ratio they change their orientation to the transmembrane disposition. So, slow rotations of the peptides seen at high P/L ratio may be attributed to dynamics of peptide molecules which are elongated along the lipid molecules of the membrane. Possible interrelation is discussed between the found topology and dynamics of peptide molecules and the membrane leakage induced by these peptides.

Molecular Magnetism and Multi-High-Frequency EPR at the National High Magnetic Field Laboratory

Stephen Hill

Department of Physics and National High Magnetic Field Laboratory, Florida State University, Tallahassee, FL32310, USA.

The EPR facilities at the National High Magnetic Field Laboratory (NHMFL) offer scientists from all over the world opportunities to use several home-built, high-field/frequency instruments with continuous coverage from ~10 GHz to 1 THz. Magnets are also available providing magnetic fields of up to 45 T. EPR performed at these extremes offers tremendous advantages for certain problems spanning diverse research fields from condensed matter physics, to chemistry, to biology. These advantages include: increased *g* anisotropy resolution; increased resolution in ENDOR; the ability to study highly anisotropic magnetic ions with large zero-field splittings; enhanced sensitivity to small samples; and the possibility to study magnetic relaxation on very short time scales. The facilities at the NHMFL offer the highest frequencies and fields for both continuous-wave and pulsed EPR anywhere in the world [1-3]. After the shameless advertising, I will provide a brief overview of the kinds of research conducted at the NHNFL, both by in-house scientists and by our external users.

The remainder of the talk will focus on applications involving molecular nanomagnets, or so-called single-molecule magnets (SMMs), which typically consist of a core of exchange-coupled transition metal ions that collectively possess a large magnetic moment per molecule. When assembled into regular crystalline arrays, the SMM unit is nominally monodisperse, i.e., each SMM in the crystal has the same spin, orientation, magnetic anisotropy and structure. This property enables detailed spectroscopies of ensembles of SMMs that have so far been lacking for other types of magnetic nanostructures [4]. Such studies have thus provided crucial insights into the quantum nature of magnetization (spin-) dynamics at the nanoscale. This talk will highlight the fascinating interplay between isotropic and anisotropic magnetic interactions that is central to the physics of SMMs, as well as emphasizing the unique insights that can be obtained using multi-high-frequency/high-field EPR. I will first focus on the factors that contribute to molecular anisotropy, followed by a discussion of the interactions that cause magnetic quantum tunneling. In particular, I will stress the value of studying families of SMMs that facilitate controllable structural modifications, thereby permitting systematic investigations of magnetization dynamics at the nanoscale [5].

[1] http://www.magnet.fsu.edu/usershub/scientificdivisions/emr/index.html
[2] G. Morley, L.C. Brunel, J. van Tol, Rev. Sci. Instrum. 79, 064703 (2008).
[3] S. Takahashi and S. Hill, Rev. Sci. Instrum. **76**, 023114 (2005).
[4] E. del Barco et al., J. Low Temp. Phys. **140**, 119-174 (2005).
[5] S. Hill et al., Dalton Trans. **39**, 4693-4707 (2010).

Can high field PELDOR be used as a standard biophysical tool to extract quantitative orientational information in SDSL studies?

Graham Smith*, Paul Cruickshank*, Duncan Robertson*, Dave Bolton*, Rob Hunter*, Hassane El Mkami*, Richard Ward***, David Norman**, Snorri Sigurdsson****, Shigeaki Nakazawa*****, Olav Schiemann***, Gunnar Reginsson***

*School of Physics and Astronomy, University of St Andrews, Scotland
**School of Life Sciences, University of Dundee, Scotland
*** School of Biology, University of St Andrews, Scotland
**** School of Chemistry, University of Iceland
*****School of Chemistry, Osaka City University, Japan

It would be very interesting if it was possible to accurately measure average relative orientations between spin label pairs, even in the presence of wide distance or angular distributions. One would then have a measurement tool to characterise small conformational angular shifts between domains or potentially distinguish interactions in multiple spin systems through their relative orientations.

These types of quantitative measurement become plausible for PELDOR measurements on nitroxide spin labels at W-band frequencies or higher, when the g-anisotropy significantly exceeds the nitrogen hyperfine couplings and becomes fully resolved. As long as the spin labels have some restriction on their mobility, it is possible to extract quantitative orientational information by making a set of PELDOR measurements that correlate different orientations by choosing different combinations of pump and probe frequencies. In our standard measurement protocol this corresponds to making a set of six PELDOR measurements, over wide measurement bandwidths, which modelling has shown can provide a highly constrained data set.

Information on the distance distribution between labels are then mainly present in the modulation of the various time-traces, whilst information on the orientational distribution are largely present in the relative modulation depths.

In practice there are two main questions: Can one have enough sensitivity to measure six high quality PELDOR data sets at high frequency on nitroxide spin labels, at standard concentrations, on a reasonable experimental time-scale?, and, Can one be confident enough in the quantitative repeatable measurement of each data set, and in the model, to extract quantitative orientational information, whilst allowing for flexibility in either the spin label or biomolecular system?

In this paper we will examine the main practical measurement issues and discuss recent experiments using a kW W-band pulse system that have given us increasing confidence that high frequency PELDOR will be an important practical biophysical measurement tool.

X-Band Rapid-scan EPR

Deborah G. Mitchell, Mark Tseytlin, Richard W. Quine, Virginia Meyer, Gareth R. Eaton, and Sandra S. Eaton,

University of Denver, Denver, Colorado 80208, USA

Rapid-scan EPR at X-Band (9.8 GHz) was investigated. The term "rapid" means that the magnetic field is scanned through resonance in a time that is short relative to relaxation times, which causes oscillations in the signal response.[1] The decay of these oscillations is dependent upon the electron spin-spin relaxation time. Thus, information about the T_2 of a sample can be extracted from simulations of rapid scan spectra. In rapid-scan EPR, the signal is detected directly, and unlike conventional slow-scan CW EPR, magnetic field modulation and phase-sensitive detection at the modulation frequency are not used. Deconvolution of the rapid scan signals results in the conventional slow scan absorption and dispersion signals.[2] For samples that are easily saturated, rapid scan EPR is advantageous. Because the magnetic field is on resonance for a very short time, the energy absorbed by the spins, for the same microwave B_1, is less than in conventional CW spectra, and the signal does not saturate as readily. The higher the rapid scan rate, the less time the magnetic field is on resonance, which allows spectra to be run at higher powers without distortion by power saturation.

Several samples were studied with rapid-scan EPR: Lithium phthalocyanine (LiPc), irradiated fused quartz, nitroxyl radicals, and α,γ-bisdiphenylene-β-phenylallyl (BDPA). LiPc was studied because of its simple Lorentzian line. Simulations of rapid scan spectra of LiPc were used to determine the T_2 relaxation time.

Rapid scans of nitroxyl radicals at increasing rates demonstrated that as the rapid scan rate increased, the linear power range increased. This enabled unsaturated spectra to be collected at much higher powers than is possible with CW EPR, which improves the signal-to-noise. Irradiated fused quartz is a sample with long spin-lattice relaxation, which is easily saturated and difficult to measure with CW EPR. Because rapid-scan EPR can be run at higher powers without saturation, undistorted rapid scan spectra of fused quartz could be collected.[3] The rapid-scan spectra were deconvolved to obtain the conventional CW spectrum.

The crystalline 1:1 BDPA:benzene complex is a stable organic radical that is commonly used in EPR and DNP experiments. Initial CW experiments using multiple particles were simulated with a Lorentzian line. However, rapid scan experiments using multiple particles resulted in spectra that could not be simulated with a single value of T_2. This observation indicated that the spectra of the BDPA samples could not be represented by a single spin packet, and that there was a distribution of relaxation times. Examination of small particles found T_2 values in air ranging from 80 to 160 ns. Thus, rapid scan EPR provided information about BDPA that was less evident in the CW experiments.

1. Joshi, J. P.; Eaton, G. R.; Eaton, S. S., Impact of resonator on direct-detected rapid-scan EPR at 9.8 GHz. *Appl Magn Reson* **2005,** *28*, 239-249.
2. Tseitlin, M.; Rinard, G.A.; Quine, R.W.; Eaton, S. S.; Eaton, G.R., Deconvolution of sinusoidal rapid EPR scans. *J. Magn Reson* **2011**, *208*, 279–283.
3. Mitchell, D. G.; Quine, R. W.; Tseitlin, M.; Meyer, V.; Eaton, S. S.; Eaton, G. R., Rapid Scan EPR of Irradiated Quartz. Submitted to Radiation Measurements.

The temperature dependence of the DNP enhancement of water protons at 3.4 Tesla

Eugeny V. Kryukov, Kevin J. Pike, Thomas K. Y. Tam, Mark E. Newton, Mark E. Smith and Ray Dupree

Department of Physics, University of Warwick, Coventry CV4 7AL, United Kingdom

There have been several recent reports of Dynamic Nuclear Polarisation (DNP) enhanced solution ^{1}H Nuclear Magnetic Resonance (NMR) investigations using modified Electron Paramagnetic Resonance (EPR) spectrometers operating at 94 GHz coupled with NMR spectrometers. [1-4] The large enhancements (ε) open up attractive perspectives for the application potential of DNP in solution NMR with macromolecular complexes. However, the magnitudes of the enhancements achieved to date have varied considerably and a lack of knowledge of the sample temperature has often precluded comparison of the different results.

In this paper we report recent measurements of the temperature dependence of the DNP enhancement of the NMR signal from water protons at 3.4 T using TEMPOL as a polarising agent. [5] For high radical concentrations (~100 mM) the leakage factor is approximately 1 and, with sufficient microwave power, the saturation factor is also approximately 1. In this situation the DNP enhancement is solely a product of the ratio of the electron and nuclear gyromagnetic ratios and the coupling factor enabling the latter to be directly determined. The use of high microwave power levels needed to ensure saturation causes rapid heating of the sample, however this does not prevent maximum DNP enhancements being obtained since the nuclear relaxation T_{1I} is very much less than the characteristic heating time at these concentrations.

The equilibrium DNP enhancement is found to vary linearly from ~ –15 at 10 °C to ~ –120 at 90 °C over the temperature range 10 °C ≤ T ≤ 100 °C. The value determined for the coupling factor at 25 °C, 0.055 ±0.003, agrees very well with molecular dynamics simulations [6] however the experimental value increases much more rapidly with increasing temperature than predicted by MD simulations. It is also shown that significant enhancements (e.g. –40 - –50) can be achieved whilst maintaining the sample temperature at 40 °C by adjusting the microwave power and irradiation time.

This work was supported by an EPSRC Basic Technology Project grant, EP/D045967.

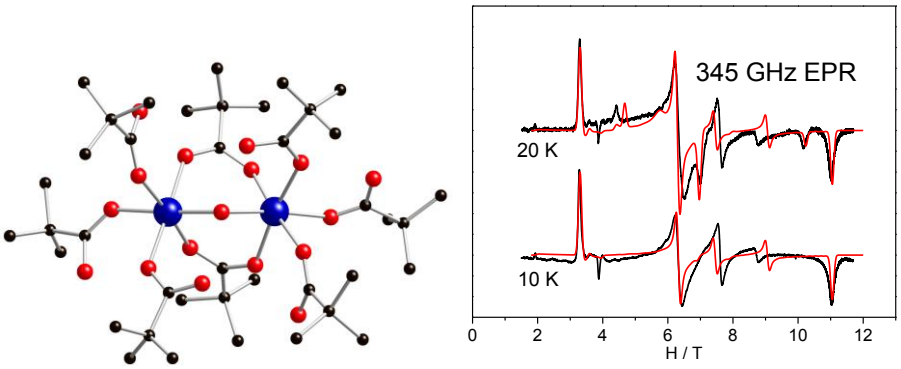
1. Hofer, P., et al., JACS, 2008. **130**(11): p. 3254-+.
2. Kryukov, E.V., et al., PCCP, 2010. **12**(22): p. 5757-5765.
3. Turke, M.T., et al., PCCP, 2010. **12**(22): p. 5893-5901.
4. Villanueva-Garibay, J.A., et al., PCCP, 2010. **12**(22): p. 5846-5849.
5. Kryukov, E.V., et al., PCCP, 2011 **13**(10): p. 4372-4380
6. Sezer, D., M.J. Prandolini, and T.F. Prisner, PCCP, 2009. **11**(31): p. 6626-6637.

A spectroscopic study of exchange-coupled orbitally-degenerate ions: six-coordinate cobalt(II) dimers.

Eric J. L. McInnes

School of Chemistry, The University of Manchester, Manchester M13 9PL, UK.

Magnetic data (including magnetic resonance) of six-coordinate cobalt(II) complexes are complicated by the orbital degeneracy of the metal ion ($^4T_{1g}$ ground term in O_h symmetry). Examples of resolved EPR spectra for *exchange-coupled*, polymetallic examples are extremely rare despite such species becoming of interest in molecular magnetism because of the potential huge magnetic anisotropies.



This lecture will detail a series of dimetallic complexes, including ferro- and anti-ferromagnetically coupled examples, with unusually well resolved EPR spectra. The spectra can be simulated using a simple model, in combination with magnetization and inelastic neutron scattering data, to give detailed insight into the low-lying electronic structure.

We are grateful to the EPSRC(UK), including for funding of the National EPR Facility at Manchester, The University of Manchester and the EC for financial support.

Magnetic anisotropy in propeller-like Single-Molecule Magnets: insights from HF-EPR

L. Sorace[*], E. Tancini[**], M.-J. Rodriguez-Douton[**], A.-L. Barra[***], R. Sessoli[*], A. Cornia[**]

[*] Department of Chemistry and INSTM, University of Florence, Sesto Fiorentino, Italy.
[**]Department of Chemistry and INSTM, University of Modena and Reggio Emilia, Modena, Italy.
[***] Laboratoire National des Champs Magnétiques Intenses - CNRS, Grenoble, France.

In the past 15 years HF-EPR spectroscopy turned out to be a key tool to characterize Single Molecule Magnets (SMMs), i.e. molecules showing slow relaxation of the magnetization at low temperature, and to provide detailed information on their magnetic anisotropy.[1] The interest toward SMMs has been intensely renewed after propeller-like $[Fe_4(L)_2(dpm)_6]$ complexes (Fe_4) were shown to retain their memory and quantum effects when grafted on surfaces, as required for applications in molecular spintronics (Hdpm is dipivaloylmethane and L^{3-} is a tripodal ligand).[2,3]

Replacement of the central Fe^{III} by a Cr^{III} in this family resulted in the heterometallic complexes $[Fe_3Cr(L)_2(dpm)_6]$. HF-EPR spectra unequivocally demonstrate that for H_3L = 2-hydroxymethyl-2-phenylpropane-1,3-diol the compound is a solid solution of Cr^{III}-centered Fe_3Cr (S = 6) and Fe_4 (S = 5) species in a 84:16 ratio. Spectral simulations indicate that the $|D|$ parameter in Fe_3Cr is considerably smaller than in Fe_4 (D = -0.179 cm^{-1} *vs* -0.418 cm^{-1}). Although SMM behaviour is retained, this leads to a lower energy barrier for magnetization reversal, as confirmed by magnetic characterization. The magnetic anisotropy in Fe_3Cr has been elucidated by preparing its Cr- and Fe- doped Ga_4 analogues. HF-EPR spectra of these systems indicate that Cr^{III} occupies the central position and Fe^{III} the two crystallographically-distinct peripheral sites, both ions featuring a positive D value. It was then concluded that the easy axis anisotropy of Fe_3Cr is entirely due to the peripheral, hard-axis type Fe^{III} ions, whose anisotropy tensors are necessarily orthogonal to the threefold molecular axis.[4] The leading contribution from peripheral ions also explains the strong correlation observed between D value and the pitch angle of the propeller structure in a series of Fe_4 derivatives.[5]

We acknowledge the support of ERC through MolNanoMas project (AdG-267746)

[1] D. Gatteschi et al. *Coord. Chem. Rev.* **2006** *250* 1514
[2] M. Mannini et al. *Nat. Mater.* **2009**, *8*, 194.
[3] M. Mannini et al. *Nature* **2010,** *468,* 417
[4] E. Tancini et al. *Chem. Eur. J.* **2010,** *16*, 10482
[5] L. Gregoli et al. *Chem. Eur. J.* **2009,** *15,* 6456

Beguiling Crystallography Cured by EPR: Rhenium Dimers with Multiple Metal Bonds

Stephen Sproules[†], Yong Yan[‡], James P. Donahue[‡], Eckhard Bill[†] and Karl Wieghardt[†]

[†]Max-Planck-Institut für Bioanorganische Chemie, Stiftstrasse 34-36, D-45470 Mülheim an der Ruhr, Germany
[‡]Department of Chemistry, Tulane University, 6400 Freret Street, New Orleans, LA 70118-5698, USA

The recognition of multiple metal-metal bonds in $[Re_2Cl_8]^{2-}$ was a landmark development in transition metal chemistry [1]. Since this defining moment, more than half the periodic table has been implicated in metal-metal bonding, and this new domain of chemistry has seen prodigious growth. Pertinent to the study of their intrinsic electronic structure, much information has been gained from reactions with $[Re_2Cl_8]^{2-}$. To that end, we have crystallographically characterised bioctahedral $[NBu_4]_3[Re_2(mnt)_5]$ (mnt^{2-} = maleonitriledithiolate) fished out of the reaction of $[Re_2Cl_8]^{2-}$ and Na_2mnt. The complex is paramagnetic ($S = {}^1/_2$) and exhibits a Re–Re distance of 2.672(1) Å indicative of a bond order of 3.5. Interestingly, its X-band EPR spectrum displays coupling to one Re ion only, a consequence of the C_2 symmetry. For comparison we recorded the EPR spectrum of isoelectronic $[Re_2(NCS)_{10}]^{3-}$ [2] because it possesses a crystallographic inversion centre. To our genuine surprise, the spectrum was consistent with an unpaired electron localised on a single Re ion, despite the presence of a Re≡Re unit (Re–Re = 2.6121(3) Å), and highlights the extent to which X-ray crystallography can be completely misleading.

---

[1] F. A. Cotton, N. F. Curtis, C. B. Harris, B. F. G. Johnson, S. J. Lippard, J. T. Mague, W. R. Robinson, J. S. Wood, *Science* **1964**, *145*, 1305.

[2] F. A. Cotton, R. Eiss, B. M. Foxman, *Inorg. Chem.* **1967**, *6*, 929.

Advanced pulsed EPR investigation of model complexes of the [FeFe] hydrogenase active site: bulky bridges *vs* bulky ligands

Özlen Ferruh Erdem[*], Lennart Schwartz[**], Matthias Stein[***], Alexey Silakov[*], Michael Singleton[****], Sascha Ott[**], Edward Reijerse[*], Marcetta Y. Darensbourg[****], and Wolfgang Lubitz[*]

[*]Max-Planck-Institute for Bioinorganic Chemistry, Mülheim a. d. Ruhr, Germany
[**]Department of Photochemistry and Molecular Science, Uppsala University, Sweden
[***]Max-Planck-Institute for Dynamics of Complex Technical Systems, Magdeburg, Germany
[****] Texas A&M University, College Station, Texas, USA

Ever since the crystallization of the [FeFe] hydrogenases [1], biomimetic and bioinspired model compounds of the active site have attracted great attention. Numerous model compounds have been reported [2], e.g. by varying the bridging dithiolate ligands, applying asymmetric ligand substitution, thus, hoping to obtain proton reduction at lower overpotentials. We have performed a detailed pulsed EPR investigation on two types of model compounds: a) Compounds structurally resembling the CO-inhibited (H_{ox}-CO) state, with a nitrogen in the bridge (*azadithiolate*-nitrogen, *adt*-N), and b) Compounds structurally resembling the H_{ox} state, having an open coordination site vacant, with a bulky bridge (2,2-dimethyl-1,3-propanedithiolate), or with a bulky ligand (N-heterocyclic carbenes). In this presentation we will use a combination of EPR, ENDOR and HYSCORE experiments as well as DFT calculations to show that i) For the H_{ox}-like complexes the spin density distribution, surprisingly, doesn't drastically change depending on having neither a bulky ligand nor a bulky bridge; ii) For the H_{ox}-CO-like complexes, the magnetic coupling parameters obtained for the amino nitrogen in the azadithiolate bridge are quite similar to those for the native system at the H_{ox} state [3] indicating that the spin density in the native binuclear sub-cluster can indeed extent up into the amino group of the bridging adt., thus providing convincing evidence for the presence of an *adt*-N in the dithiolate bridge of the active site of native [FeFe] hydrogenase [4]. The confirmation of this amino function in the bridge is of relevance to the proposed mechanism of [FeFe] hydrogenases since it may act as proton donor/acceptor.

[1] Peters, J. W.; Lanzilotta, W. N.; Lemon, B. J.; Seefeldt, L. C. *Science,* **1998***, 282*, 1853; J. C. Fontecilla-Camps, P. Amara, C. Cavazza, Y. Nicolet, A. Volbeda, *Nature*, **2009**, *460*, 814.
[2] Tard, C.; Pickett, C. J. *Chem. Rev.,* **2009**, *109*, 245.
[3] Silakov, A.; Wenk, B.; Reijerse, E.; Lubitz, W. *Phys. Chem. Chem. Phys.*, **2009**, *11*, 6592.
[4] Erdem, O. F.; Schwartz, L.; Stein, M.; Silakov, A.; Kaur-Ghumaan, S.; Huang, P.; Ott, S.; Reijerse E.; Lubitz, W. *Angew. Chem. Int. Ed.*, **2011**, *123*, 1475.

New developments in EPR and DNP

and application to biomolecular research

Thomas Prisner

Institute of Physical and Theoretical Chemistry and

Center for Biomolecular Magnetic Resonance , Goethe University Frankfurt

This talk will give an overview of our recent methodological work in the field of pulsed and high-field EP, as well as high-field DNP, illustrated by some new applications to determine structure and dynamics of biological macromolecules and complexes in solution, lipids and cells.

1. Marina Bennati & Thomas F. Prisner; New developments in high field electron paramagnetic resonance with applications in structural biology, Reports on Progress in Physics, 68, 411-448 (2005).
2. O. Schiemann, T. F. Prisner Long Range Distance Determinations in Biomacromolecules by EPR Spectroscopy Quart.Rev. Biophys., 40, 1-53 (2007)
3. O. Schiemann, N. Piton, J. Plackmeyer, B.E. Bode, T.F. Prisner & J.W. Engels; Spin labeling of oligonucleotides with the nitroxide TPA and use of PELDOR, a pulse EPR method, to measure intramolecular distances Nat. Protoc., 2, 904-923 (2007).
4. T. Maly, K. Zwicker, A. Cernescu, U. Brandt, T. F. Prisner New pulsed EPR methods and their application to characterize mitochondrial complex I Biochim. Biophys. Acta 1787, 584-592
5. M. J. Prandolini, V. P. Denysenkov, M. Gafurov, B. Endeward, T. F. Prisner High-Field Dynamic Nuclear Polarization in Aqueous Solutions J. Am. Chem. Soc. 131, 6090-6092 (2009)
6. V. Denysenkov, M. J. Prandolini, M. Gafurov, D. Sezer, B. Endeward, T. F. Prisner Liquid state DNP using a 260 GHz high power gyrotron Phys.Chem.Chem.Phys. 12, 5786-5790 (2010)
7. S. Lyubenova, T. Maly, K. Zwicker, U. Brandt, B. Ludwig, T. F. Prisner Multi-Frequency Pulsed EPR on Metalloproteins Acc. Chem. Res. 43, 181-189 (2010)
8. Ivan Krstić, Robert Hänsel, Olga Romainczyk, Joachim W. Engels, Volker Dötsch, Thomas F. Prisner In-cell Long Range Distance Measurements on Nucleic Acids by Pulsed EPR Spectroscopy Angewandte Chemie Int. Edit. (2011)

Exploring the energy landscape and dynamic modes of a protein with site directed spin labeling

Wayne L. Hubbell

Jules Stein Eye Institute and Department of Chemistry and Biochemistry
University of California, Los Angeles CA 90095

Alan Cooper pointed out many years ago that because of their small size and finite compressibility, protein molecules must undergo substantial volume and structural fluctuations [1]. Thus, it was not a total surprise when NMR was subsequently able to show that at least some proteins in solution display a manifold of conformational substates at equilibrium, with exchange lifetimes on the order of μs – ms. On the other hand, it was a surprise that evolution has apparently taken advantage of the different conformational substates (perhaps a continuum of states) to achieve remarkably complex networks of functional protein-protein interactions. Moreover, the fast kinetics of protein-protein interactions *via* a diffusive encounter with lifetime ≈ ns may also rely on dynamic disorder on this timescale in the recognition sequence.

Thus, to explore the role of molecular flexibility in protein function it is essential to have experimental tools to identify the existence of conformational substates, to measure their exchange rate and to detect dynamically disordered sequences involved in protein-protein recognition. NMR relaxation methods can provide such information on an atomic level for small proteins in solution, but face significant challenges in the study of more complex systems, including membrane proteins in their native environment. Site Directed Spin Labeling (SDSL) provides an alternative approach, not limited by the size and complexity of the system, with significant advantages of sensitivity and time scale. In recent studies, the principles underlying the application of line shape analysis [2], pulse saturation recovery [3], osmotic perturbation [4], and high pressure [5] in SDSL studies of protein dynamics were described. In this report, the first application of these strategies to map the global energy landscape and flexibility of a protein will be discussed with an emphasis on the technology and the feasibility of general applications.

[1] Cooper A (1984) Prog. Biophys. Mol. Biol. 44:181.
[2] Columbus L, Hubbell W (2004) Biochemistry 43:7273.
[3] Bridges M, Hideg K, Hubbell W (2010) Appl. Magn. Reson. 37:363.
[4] Lopez C et al (2009) Protein Sci. 18:1637.
[5] McCoy J, Hubbell W (2011) PNAS, 108:133.

Site-directed spin labelling EPR – targeting protein structure, dynamics and function

Johann P. Klare, Heinz-Jürgen Steinhoff

Physics Department, University of Osnabrueck, Barbarastr. 7, 49076 Osnabrueck, Germany, *johann.klare@uos.de*

Site-directed spin labelling (SDSL) EPR spectroscopy is a powerful tool to elucidate the structural and dynamic properties of biomolecules and biomolecular systems. It offers a wide range of techniques, from the "classical" cw methods – providing information about the spin label side chain mobility, solvent accessibility and the polarity of its immediate environment, to pulse EPR methods like ESEEM and PELDOR/DEER. The latter technique, especially since commercial instruments became available, has become one of the most popular tools in structure-function studies by EPR during the last decade.

This presentation gives an overview on selected examples from work on different proteins and protein complexes, membrane proteins as well as soluble proteins undergoing dimerization, and an approach where, instead of labelling the protein under investigation, spin labelled ligands/inhibitors of a protein are used.

Each example presented here highlights specific techniques used to address the respective questions:

• A "nitroxide scanning" study, where mobility, accessibility and polarity data obtained from cw EPR experiments reveal detailed insights into a specific motif of a membrane-integral symporter.

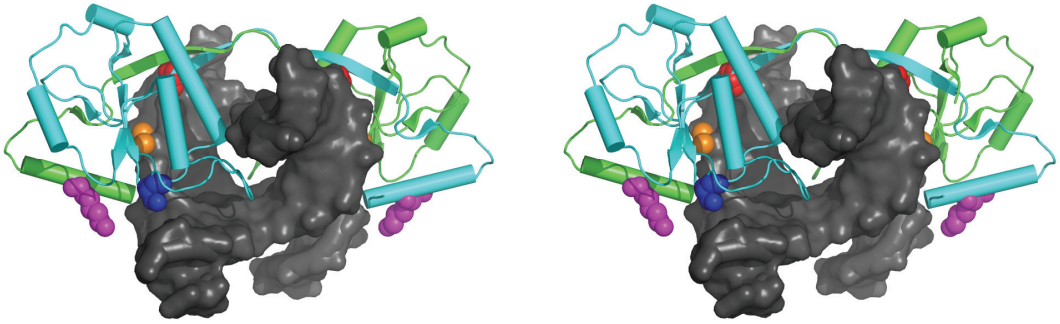
• Inter-spin distance measurements by cw- and pulse EPR to unravel the overall structure of a homodimeric GTPase, the human guanylate-binding protein 1, as well as conformational changes in the protomers taking place upon dimerization.
• Application of spin labelled ligands/inhibitors of two large membrane protein complexes, the V-ATPases from *M. Sexta* and S. *cerevisiae*, to determine the modes of binding, gain insights into the mechanisms of inhibition and, furthermore, reveal the stoichiometry of this protein complex.

A Site Specific Spin Labeling and EPR Study of a complex between Endonuclease I and DNA-4-way junction

Richard Ward[*], Alasdair D. J. Freeman[*], Hassane El-Mkami[**], David M. J. Lilley[*], David G. Norman[*]

[*] Cancer Research UK Nucleic Acid Structure Research Group Department of Biochemistry MSI/WTB Complex, The University of Dundee, Dundee DD1 5EH, UK.
[**]University of St. Andrews, School of Physics & Astronomy, North Haugh, St Andrews, Fife KY16 9SS, UK

Recognition and manipulation of the DNA four-way (Holliday) junction (DNA-4WJ) is central to the process of homologous recombination, which in turn is important in the generation of genetic diversity and is crucial to some DNA damage repair processes. Endonuclease I (endoI), from bacteriophage T7[1], selectively binds and cleaves DNA four-way junctions (DNA-4WJ). The crystal structure of endoI has been solved[2] both in its wild type form and also as a cleavage-inactive mutant bound to an immobile DNA-4WJ[3]. The protein dimer is extremely tightly bound and forms two globular domains that are joined by a β-strand bridge, each globular domain containing an active site that is contributed to by both monomeric units. The N-terminal helix(α1) of endoI has been seen in crystal structures of both the isolated protein and in complex with a DNA junction. The N-terminal 16 residues however did not show enough electron density to be fitted and the B-values of the α1 were particularly poor in the junction complex. We have used site-specific spin labeling in conjunction with EPR distance measurements (PELDOR[4 5] to monitor the binding of a DNA-4WJ to endoI. In addition we have examined the structure of wild type, and catalytically inactive, endoI, alone and in complex with a DNA-4WJ. We demonstrate the presence of hitherto undefined metastable conformational states within endoI and describe how the equilibrium in conformational states can be influenced by DNA-junction binding or mutations that disrupt the active sites of the protein and lead to enhanced instability in the N-terminal helix.



Stereo view of endoI bound to DNA-4WJ (PDB code 2PFJ).

(1) Center, M. S.; Richards.Cc *Journal of Biological Chemistry* **1970**, *245*, 6292.
(2) Hadden, J. M.; Convery, M. A.; Declais, A. C.; Lilley, D. M.; Phillips, S. E. *Nat Struct Biol* **2001**, *8*, 62.
(3) Hadden, J. M.; Declais, A. C.; Carr, S. B.; Lilley, D. M.; Phillips, S. E. *Nature* **2007**, *449*, 621.
(4) Milov, A. D.; Salikohov, K. M.; Shirov, M. D. *Fizika Tverdogo Tela* **1981**, *23*, 975.
(5) Pannier, M.; Veit, S.; Godt, A.; Jeschke, G.; Spiess, H. W. *Journal of Magnetic Resonance* **2000**, *142*, 331.

PELDOR applications for the spatial distribution studies of paramagnetic centers.

A.D.Milov and Yu.D.Tsvetkov

Institute of Chemical Kinetics and Combustion, Novosibirsk, Russian Federation

PELDOR spectroscopy makes it possible not only to obtain data on distances in radical pairs or complexes, but also to study the spatial configuration of spin systems. The theory of dipolar interactions and the calculation methods are well developed. Therefore information on the geometry of spatial distribution of paramagnetic centers can be extracted from the law that describes the kinetics of PELDOR signal decay.

In this report we shall present examples on different types of spatial distributions including the case of charged ion-radicals (Fremy salt). It is shown that as compared with neutral radicals, in the case of the ions-radical, the PELDOR decay deviates from the exponential one. The observed decay low for the ion-radicals corresponds to electrostatic repulsion effect for the charged particles. It is shown that the relative space distribution of ion-radical based on the Debye-Huckel theory gives a satisfactory description of the dipole-dipole spin phase relaxation for charged radicals.

An example is provided by the results of calculations of the kinetics of PELDOR signal decay for the spin labels localized on the cell membrane or within the membrane.

Spectral information from unexpected places: tumor physiology with in vivo EPR imaging

Howard J. Halpern,[a,b] ,Boris Epel,[a,b] Kang-H. Ahn,[a,b] Chad R. Haney,[a,b] Eugene D. Barth,[a,b] Martyna Elas, [a,b,c] V.S. Subramanian,[a,b] Brandi Butler, [a,b] Chanel Li, [a,b] Marta A. Zamora,[d] Jonathan N. River,[d] Gregory S. Karczmar,[d] Helena J. Mauceri,[b] Ralph R. Weichselbaum,[b] Colin Mailer,[a,b] Charles A. Pelizzari,[a,b] Gareth R. Eaton,[f] George A. Rinard,[f] Richard Quine,[f] and Victor Tormyshev[g]

[a]Center for EPR Imaging In Vivo Physiology; [b]Department of Radiation Oncology, University of Chicago, Chicago IL 60637 USA; [c]Molecular Biology, Jagiellonian University, Cracow, Poland; [d]Department of Radiology, University of Chicago, Chicago IL 60637, USA; [f]Chemistry and Biochemistry, University of Denver, Denver CO, USA; [g]Novosibirsk Institute for Organic Chemistry, Novosibirsk, Siberia, Russia.

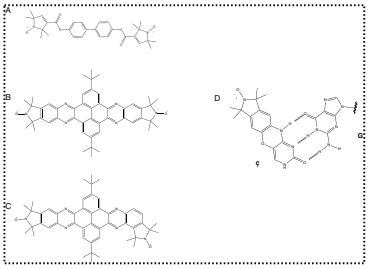
Howard Halpern: MC1105 Dept Rad. Oncology, U. Chicago Med. Ctr, 5841 S. Maryland Ave, Chicago IL 60637 USA. FAX: 1-773-702-5940, h-halpern@uchicago.edu

Electron paramagnetic resonance (EPR) oxygen images (EPROI) using trityl spin probes have demonstrated the ability to 1) reproduce gold standard pO_2 *measurements* in the tumors of living mice 2) to add significant information to radiation dose in the prediction of tumor cure 3) provide a major image based determinant in the prediction of radiation cure of mouse tumors treated to a single dose and 4) image rapid post radiation changes in tumor oxygenation that help explain the effectiveness of large hypofractionated radiation treatements. While these biologic measurements have progressed, so has the EPROI technique. We have progressed from 45 minute continuous wave EPROI to pulsed, electron spin echo (ESE) images requiring 10 minutes for essentially the same information. We have modified the ESE technique to adapt to the biologic needs of the experiment including novel resonator designs that allow rapid access of the mouse anatomy to both the needs of imaging and the need to radiate without significant perturbation of the tumor. We have optimized the information per unit time with Zeeman offset ESE image acquisition and with repetition time saturation corrections that allow high repetition rate without compromise of the transverse relaxation time measurement accuracy. We have, in the process discovered significant trityl self broadening or transverse relaxation effect due to excluded volume and high local concentration. This, in turn, is massively minimized using T_1 imaging with an inversion recovery initial pulse, since the magnetization energy is only redistributed among the spin probes through self interaction, not affecting the longitudinal, energy relaxation. Significantly more accurate EPROI are demonstrated.

PELDOR measurements on nitroxide biradicals and non-covalently spin labelled DNA using Hiper.

Gunnar W. Reginsson[*], Sandip Shelke[†], Robert Hunter[§], Graham Smith[§], Snorri Th. Sigurdsson[†], Olav Schiemann[*]

[*] University of St Andrews, Biomedical Sciences Research Complex, Centre of Magnetic Resonance, North Haugh, KY16 9ST, St Andrews, UK.
[†] University of Iceland, Science Institute, Dunhaga 3, 107 Reykjavik, Iceland.
[§] University of St Andrews, School of Physics and Astronomy, Centre of Magnetic Resonance, North Haugh, KY16 9ST, St Andrews UK.



PELDOR (pulsed electron double resonance) together with site directed spin labelling has become a valuable technique to measure nanometer distances on biomolecules with high precision. In addition to distances PELDOR can also be used to determine the mutual orientation of spin centers. If the attached spin labels are rigid relative to the biomolecule, orientations and conformational distributions of domains can be determined. Using a home-built W-band spectrometer (Hiper) [1] we have performed orientation selective PELDOR measurements on semi-flexible (A) and exchanged coupled rigid nitroxide biradicals (B,C) [2]. Simulating the PELDOR data we have been able to measure quantitatively the molecular flexibility, angles between spin labels and the anisotropic exchange coupling in these model systems. To demonstrate the application of orientation selective PELDOR measurements to biological samples we have used DNA, non-covalently spin labelled with a cytosine analogue (D) [3]. From the orientation selective W-band PELDOR measurements and simulations, we are able to probe the inherent dynamics of DNA from spin label orientations and distances.

[1] P. A. S. Cruickshank, D. R. Bolton, D. A. Robertson, R. I. Hunter, R. J. Wylde, G. M. Smith. A kilowatt pulsed 94 GHz electron paramagnetic resonance spectrometer with high concentration sensitivity, high instantaneous bandwidth, and low dead time. Rev. Sci. Instrum. 2009. 80. 103102

[2] D. Margraf, P. Cekan, T. Prisner, S. Th. Sigurdsson, O. Schiemann (2009). Ferro- and antiferromagnetic exchange coupling constants in PELDOR spectra. Phys. Chem. Chem. Phys. 11: 6708-6714.

[3] S. A. Shelke, S. Th. Sigurdsson (2010). Noncovalent and Site-Directed Spin Labeling of Nucleic Acids. Angew. Chem. Int. Ed. 49 (43): 7984-7986.

Viewing the Human Pancreatic Lipase lid opening by Site Directed Spin Labeling combined with CW and pulsed EPR spectroscopy

S. Ranaldi[*], M. Woudstra[*], H. Vezin[***], F. Carrière[**], B. Guigliarelli[*], A. Fournel[*], V. Belle[*]

[*] BIP Laboratory CNRS UPR 9036 and Aix-Marseille University, Marseille, FR
[**] EIPL Laboratory CNRS UPR 9025 and Aix-Marseille University, Marseille, FR
[***] LASIR Laboratory UMR 8516, Lille University, Villeneuve d'Ascq, FR

Human pancreatic lipase (HPL) is the key enzyme for fat digestion. Soluble in water whereas its natural substrates (triglycerides) are insoluble, HPL carries out an interfacial catalysis involving a conformational change. X-ray crystallography revealed two different conformations of HPL differing from the position of a lid covering the active site (closed conformation) or not (open conformation) [1a, 1b].

In view of achieving a dynamic description of the opening of the lid of the HPL in solution, as well as to unveil the possible coexistence of several lid conformations, we used site-directed spin labeling coupled to EPR spectroscopy, a powerful technique to study conformational changes in proteins [2].

In a first approach, a paramagnetic probe (MTSL nitroxide) was covalently bound to the HPL lid and EPR spectroscopy was used to monitor the conformational change induced by the presence of its physiological partners (colipase and bile salts). In this study, two specific EPR spectral shapes were assigned to the closed and open conformation of HPL. This assignment allowed us to analyze the influence of each physiological partner on the lid opening, to demonstrate the reversibility of this process [3] and to reveal particular structural changes induced by lowering the pH [4].

In a second approach, a strategy of double spin labeling was applied for measuring the amplitude of the lid opening *via* inter-spin distance measurements by Double Electron-Electron Resonance (DEER) experiments. The inter-spin distances measured were found to be in good agreement with those estimated from the known X-ray structures, demonstrating that HPL conformations in solution are similar to those observed in the enzyme crystals [5]. The perspective of this work is to investigate the lid opening process when the HPL binds at the lipid-water interface.

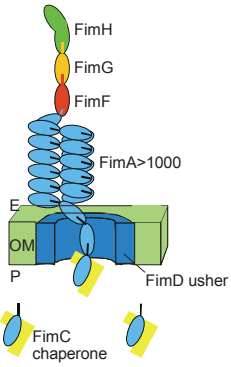
[1] **a**. Winkler F.K. *et al*, *Nature*, **343**, 771-774, **1990**
b. Van Tilbeurgh H. *et al*, *Nature*, **362**, 814-820, **1993**.
[2] Hubbell W.L. *et al*, *Nat Struct Biol*, **7**, 735-739, **2000**.
[3] Belle V. *et al*, *Biochemistry*, 46, 2205-14, **2007**.
[4] Ranaldi S. *et al*, *Biochemistry*, 48, 630-638, **2009**.
[5] Ranaldi S. *et al*, *Biochemistry*, 49, 2140-49, **2010**.

Polymerisation mechanism at the outer membrane usher of Type 1 pili from *E.coli* investigated by SDSL-EPR

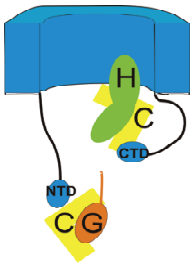
Katharina F. Pirker[*], William J. Allen[**], Micha B. A. Kunze[*], Gabriel Waksman[*,**] and Christopher W. M. Kay[*]

[*] Institute of Structural & Molecular Biology, University College London, Gower Street, London WC1E 6BT, UK.
[**] Institute of Structural & Molecular Biology, Birkbeck College, London WC1E 7HX, UK.

Bacterial infection by uropathogenic *Escherichia coli* (UPEC) is the primary cause of urinary tract infections in Europe and North America and affects many individuals; especially women. There is an increase in resistance to antibiotics by these bacteria and studies of the onset of bacterial infection are gaining importance. Infection of host cells by these bacteria is mediated by fibers (also called *pili*) which are assembled by the chaperone-usher (CU) pathway (Scheme 1). The surface fiber type 1 *pili* are important attachment devices that target UPEC to the bladder epithelium and are encoded by the *fim* gene cluster (*fimA-I*). Type 1 *pili* are thus major virulence factors in the onset of cystitis. These pili are assembled by the chaperone-usher pathway, involving a periplasmic chaperone (FimC) and an outer-membrane assembly platform, the usher (FimD). Chaperone-subunit complexes are recruited by the N-terminal domain (NTD) of the usher and the usher orchestrates the ordered polymerisation (via donor-strand-exchange reaction, DSE) and secretion of subunits.



Scheme 1



Scheme 2

We are using site-directed spin labelling (SDSL) in combination with continuous wave (cw) and pulsed electron paramagnetic resonance (EPR) spectroscopy to investigate the polymerisation mechanism at the usher. Starting with the usher-chaperone-subunit complex FimD:FimC:FimH, the next chaperone-subunit FimC:FimG is added. Spin labels are positioned on the C-terminal domain (CTD) of the usher FimD and the chaperone FimC of FimC:FimG. By measuring distances between spin labels, we could show by EPR that after the DSE reaction, the subunit FimG binds to the CTD. This result is comparable to the recently solved crystal structure of FimD:FimC:FimH where the previous subunit FimH is bound to the CTD of the usher. The involvement of the NTD in the recruitment of the subunits is still not understood [1]. We are now investigating the intermediate state of the FimD:FimC:FimH:FimG complex, right after the addition of the subunit FimG and before it undergoes DSE, i.e. binding to the previous subunit FimH. A plausible intermediate state is suggested in the Scheme 2.

[1] Phan et al., *"Crystal structure of the FimD usher bound to its cognate FimC:FimH substrate"*, (2011) submitted.

MD simulation of spin labelled yeast iso-1 cytochrome *c* protein: a tool for predicting the EPR

Egis Kuprusevicius, Gaye F. White, Andrew J. Thomson* and Vasily S. Oganesyan*

School of Chemistry, University of East Anglia, Norwich, NR4 7TJ, United Kingdom

Nitroxide spin labels are widely used by both CW and pulsed EPR in order to probe local structure and dynamics, monitor conformational changes and measure long distances and orientations in complex molecular systems such as proteins and protein-protein complexes, DNA/RNA, polymers, cell membranes and nanostructures.

Recently we have reported a novel general methodology for prediction of motional EPR spectra from single Molecular Dynamics (MD) trajectories of spin labels [1]. It has been successfully applied to different partially disordered systems [2]. The advantage of using MD trajectories at the atomistic level for predicting EPR is the ability to extract and inspect separately the different motional contributions to the overall complex dynamics of the label.

Here we present the application of our novel MD-EPR simulation approach to the analysis of spin labelled *Saccharomyces cerevisiae* iso-l-cytochrome *c* containing low spin heme. For the first time MD simulations have been used to predict both multi-frequency CW EPR spectra and the relaxation enhancement of the probe [3] by the low spin Fe(III) heme, providing detailed characterisation of both the dynamics and orientation of the spin probe and an estimate of the distance between the Fe(III) heme and the spin label [4].

Both CW and pulsed EPR data simulated from MD on spin labelled cytochrome *c* are in excellent agreement with experimental measurements. It is demonstrated that the protein backbone contribution is crucial for the motionally averaged EPR line shape while rotameric dynamics of the tether of the label remain constrained resulting in narrow nitroxide-Fe heme distance distribution. Interestingly, our study shows that motionally averaged EPR spectra do not automatically imply a flexible position for the label. Thus sites which are associated with high mobility in EPR spectra can be as suitable for distance measurements as restricted sites.

The computational protocol and findings reported constitute further steps in bringing together theory and experiment for the development of “computer engineering” of spin labelled proteins prior to EPR measurements.

[1] V.S. Oganesyan, *J. Magn. Reson.*, **2007**, 188, 196; V.S. Oganesyan, *Phys. Chem. Chem. Phys.*, **2011**, DOI: 10.1039/C0CP01068E.

[2] E. Kuprusevicius, R. Edge, H. Gopee, A.N. Cammidge, E.J.L. McInnes, M.R. Wilson, and V.S. Oganesyan, *Chem. Eur. J.*, **2010**, 16, (38), 11558.; E. Kuprusevicius, G.F. White and V.S. Oganesyan, *Faraday Discuss*., **2011**, 148, 283.

[3] Oganesyan V.S., White G.F., Field S., Marritt S., Gennis R.B., Yap L.L. and Thomson A.J., *J. Biol. Inorg. Chem.*, **2010**, 15, 1255.; [4] E. Kuprusevicius, G.F. White, A.J. Thomson and V.S. Oganesyan, *Chem. Eur. J.*, **2011,** submitted

Spinach – a software library for simulation of spin dynamics in large spin systems

H.J. Hogben[1], M. Krzystyniak[2], G.T.P. Charnock[2], P.J. Hore[1], Ilya Kuprov[2,*]
[1]*Chemistry Department, University of Oxford, South Parks Road, Oxford, UK.*
[2]*Oxford e-Research Centre, University of Oxford, 7 Keble Road, Oxford, UK.*

We introduce a software library incorporating our recent research into efficient simulation algorithms for large spin systems. Liouville space simulations (including symmetry, relaxation and chemical kinetics) of most liquid state NMR experiments on 40+ spin systems can now be performed without effort on a desktop workstation. Much progress has also been made with improving the efficiency of ESR, solid state NMR and Spin Chemistry simulations.

The functionality available at the time of writing includes:

- Low-dimensional matrix representations for spin operators in large spin systems that enable the simulation of magnetic resonance experiments on systems previously considered too big for any practical simulations.
- Generalized symmetry module (any number of groups of equivalent spins of any quantum number).
- Krylov subspace based time propagation routines that avoid matrix exponentiation.
- Generalized rotation module and a Lebedev powder integrator.
- Generalized Redfield relaxation theory module, supporting all types of magnetic resonance spectroscopy (NMR, ESR, DNP, Spin Chemistry, *etc.*).
- Analytical derivatives and optimal control (BFGS-GRAPE) module.
- Functions for multi-grid parallel soft pulses and (algebraic) decoupling.
- Functions for the simulation of magnetochemical experiments.
- Functions for the simulation of common NMR experiments (COSY, DQF-COSY, NOESY, HSQC, HMQC, HETCOR, *etc.*), basic ESR experiments (ESEEM, ENDOR, *etc.*) as well as building blocks for the writing of user-specified experiment simulations.
- An example set with over 50 well-commented sample simulations spanning all areas of Magnetic Resonance spectroscopy.

Spinach is an open-source Matlab library available at http://spindynamics.org.

[1] H.J. Hogben, M. Krzystyniak, G.T.P. Charnock, P.J. Hore, I. Kuprov, *J. Magn. Reson*, **2011** (208) 179-194.
[2] I. Kuprov, *J. Magn. Reson*, **2011**, 10.1016/j.jmr.2010.12.004.

Spin Ensemble Entanglement

Stephanie Simmons*,[1] Vasileia Filidou*,[1] Richard Brown,[1] Mike L. W. Thewalt,[2] Kohei M.Itoh,[3] Steven D. Karlen,[4] Harry Anderson,[5] Feliciano Giustino,[1] and John J. L. Morton[1, 6]

[1]Department of Materials, Oxford University, Oxford OX1 3PH, UK
[2]Department of Physics, Simon Fraser University, Burnaby, BC, Canada
[3]School of Fundamental Science and Technology, Keio University, Yokohama, Japan
[4]Department of Chemistry and Argonne-Northwestern Solar Energy Research (ANSER) Center, Evanston, IL 60208-3113, USA
[5]CRL, Department of Chemistry, University of Oxford, Oxford OX1 3TA, UK
[6]CAESR, Clarendon Laboratory, Oxford University, Oxford OX1 3PU, UK

Entanglement is one of the most technologically important quantum phenomena and its controlled creation brings us a step closer to the realisation of a quantum computer. Here we report two methods for creating on-demand ensemble entanglement. The first uses permanent electron spins coupled to donor nuclear spins in isotopically engineered phosphorus-doped silicon to generate nuclear-electronic entanglement. For this, we combined high field/low temperature electron spin resonance (3.4 T, 2.9 K) with hyperpolarisation of the ^{31}P nuclear spin to obtain an initial state of sufficient purity to create a non-classical, inseparable state. The second method uses a transient photo-excited electron spin to in the phosphine oxide fullerene to generate pseudo nuclear-nuclear entanglement. To accomplish this, detailed characterisation studies into the inter-qubit coupling and relaxation kinetics were performed alongside first principle density functional calculations. For both experiments, the state's density matrix was extracted using quantum state tomography.

*both authors contributed to, and will present, this work

Spin Dynamics in Molecular Magnets

C. J. Wedge, S. J. Blundell & A. Ardavan.
CÆSR, Clarendon Laboratory, University of Oxford, OX1 3PU, UK

G. A. Timco, F. Tuna, E. J. L. McInnes & R. E. P. Winpenny.
Department of Chemistry, University of Manchester, M13 9PL, UK

The proposal that molecular magnets could be used as solid state qubits [1] has stimulated great interest in these systems during the past decade, with further impetus provided by the first measurements of phase coherence times [2]. It has often been stated that the flexibility in their synthesis should allow engineering of molecular magnet systems with desirable properties [3], and indeed individual Cr_7Ni molecular magnets have been both linked to provide coupled qubits [4] and deposited onto surfaces in preparation for single molecule measurements [5]. Here we present a systematic investigation into how the various chemical components of Cr_7Ni molecular magnets influence their relaxation properties, in what is to the best of our knowledge the first study to substantiate the claim that synthetic flexibility allows tunability of magnetic properties. We provide evidence for the dominant decoherence pathways in these systems and have applied this information to extend the phase coherence times to exceed 3 μs in a protonated system and to reach 5.6 μs in a deuterated system.

[1] M. N. Leuenberger & D. Loss, *Nature*, **410**, 789–793 (2001).

[2] A. Ardavan, O. Rival, J. J. L. Morton, S. J. Blundell, A. M. Tyryshkin, G. A. Timco, & R. E. P. Winpenny, *Phys. Rev. Lett.*, **98**(5), 057201 (2007).

[3] C. M. Ramsey, E. del Barco, S. Hill, S. J. Shah, C. C. Beedle & D. N. Hendrickson, *Nat. Phys.*, **4**, 277–281 (2008).

[4] G. A. Timco, S. Carretta, F. Troiani, F. Tuna, R. J. Pritchard, C. A. Muryn, E. J. L. McInnes, A. Ghirri, A. Candini, P. Santini, G. Amoretti, M. Affronte & R. E. P. Winpenny, *Nat. Nanotechnol.*, **4**, 173–178 (2009).

[5] V. Corradini, F. Moro, R. Biagi, V. De Renzi, U. del Pennino, V. Bellini, S. Carretta, P. Santini, V. A. Milway, G. Timco, R. E. P. Winpenny & M. Affronte, *Phys. Rev. B*, **79**(14), 144419 (2009).

Radical transfer reactions mediated by heme proteins and their prevention

Magdalena A. Lam*, David I. Pattison*, Kathryn E. Fairfull-Smith**, Trine K. Dalsgaard ***, Steven E. Bottle**, Michael J. Davies*

* The Heart Research Institute, 7 Eliza Street, Newtown, Sydney, Australia
** School of Physical and Chemical Sciences, QUT, Brisbane, Australia
*** Dept, of Food Science Aarhus University, Tjele, Denmark

Radicals are generated in biological systems by multiple processes, and there is considerable evidence for such reactions being a key process in food spoilage, in aging and human disease. Oxidant formation by heme peroxidases (e.g. myeloperoxidase) and other heme proteins (e.g. hemoglobin, myoglobin) has been implicated in multiple human inflammatory diseases including heart disease, rheumatoid arthritis, neurodegenerative conditions and some cancers. There is therefore considerable interest in both how such damage occurs, and its prevention.

We have examined potential radical transfer reactions between H_2O_2- activated heme proteins and other biological targets using multiple techniques including EPR spectroscopy, pulse radiolysis and product analysis. The data obtained indicate that these reactions are a significant source of biological damage, but that the reactions involved are dependent on the protein structure and the nature of the target.

The potential inhibition of these reactions by nitroxide radicals has been examined. These species have multiple effects including reaction with specific amino acid and protein-derived radicals, including those generated from tyrosine and tryptophan; absolute rate constants for some of these reactions have been determined by pulse radiolysis and are in the range 10^6 -10^8 M^{-1} s^{-1}. These values are comparable, or higher, than those for "traditional" antioxidants (e.g. ascorbic acid and glutathione).

Nitroxides can also act as inhibitors of oxidant formation by interfering with the catalytic cycles of some peroxidases. In the case of myeloperoxidase, HOCl formation from H_2O_2 and Cl^-, was inhibited by 4-aminoTEMPO with an IC_{50} value of ~ 1 μM. Studies with activated human neutrophils, a major source of myeloperoxidase in vivo, yielded an IC_{50} of ~ 6 μM. Structure-activity relationships have been examined for a range of nitroxides, and some of the factors that control this process have been determined. These data indicate that nitroxides have potential as inhibitors of oxidant-mediated damage in inflammatory diseases.

Key references:

1) Davies, M.J., Biochim. Biophys. Acta, (2005) 1703, 93-109.
2) Lam, M.A.; Pattison, D.I.; Bottle, S.E.; Keddie, D.J.; Davies, M.J., Chem. Res. Toxicol. (2008) 21, 2111-2119.
3) Rees, M.D.; Bottle, S.E.; Fairfull-Smith, K.E.; Malle, E.; Whitelock, J.M.; Davies, M.J., Biochem. J. (2009) 421, 79-86.

Investigating UVA-induced DNA-photosensitiser radical combinations in DNA-riboflavin; and relevance to cell nuclei

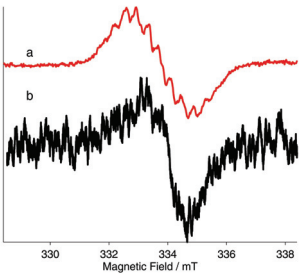
Rachel M. Haywood[*], Nick Kassouf[*], Christopher W. M. Kay[**]

[*]RAFT Institute, Mount Vernon Hospital, Northwood, Middlesex, HA6 2RN, UK.
[**]Institute of Structural & Molecular Biology and London Centre for Nanotechnology, University College London, Gower Street, London WC1E 6BT, UK.

In this work we have examined in detail the EPR signal generated upon UVA-visible irradiation of DNA-riboflavin, which is intense in the absence of oxygen and proposed to consist of DNA and riboflavin radicals [1].

Anaerobic DNA-riboflavin combinations in acidic, neutral and alkali pH were exposed either to UVA-vis or blue laser light, and CW-ESR spectra obtained on both Jeol FA100 and Bruker EMX spectrometers at room temperature and in frozen solution. At low temperature a broad resonance lacking hyperfine splitting was detected, which differed from the spectrum of a protein-bound neutral flavin radical. A pulsed ENDOR spectrum further confirmed the predominance of flavin radicals, but due the similar structures of Guanosine (the most readily oxidised DNA base) and riboflavin, distinction of these radicals is complicated. At room temperature, decreasing the acidic DNA concentration from 200 to 12 mg/ml (at constant 1mM riboflavin) resulted in increased resolution of hyperfine splitting (a), which was abolished in D_2O suggesting this to result from flavin radicals protonated at N(5) [2]. Comparable irradiation of EDTA-riboflavin (known to generate flavin radicals) resulted in the detection of a weak broad signal suggesting the flavin radical to be relatively unstable at acidic pH, but stabilised by DNA. Similar stabilisation of flavin radicals by DNA was observed at neutral pH, although the spectra differ in line shape and width presumably due to double-stranded compared to denatured DNA at low pH. At high pH, where DNA is also denatured, the spectra are broad with well-resolved hyperfine splitting, and similar spectra were obtained in irradiated Guanosine-riboflavin systems.

We suggest that the broad spectra obtained in irradiated DNA-riboflavin systems comprise both DNA and flavin radicals, which cannot be readily distinguished due the association of flavin radicals with DNA. In isolated (DNA rich) nuclei of cells, however, a broad spectrum tentatively assigned to Guanosine$^{+\cdot}$ was obtained (b), which is not complicated by the dominance of photosensitiser radicals.



Our findings are discussed in relation to studies of DNA damage in biological systems.

[1] Haywood R *et al. Photochem Photobiol*. 2011, 87:117-30.

[2] Kay CWM *et al. Biochemistry*.1999; 38 (51):16740-8.

Applications of EPR Spectroscopy in Pharmaceutical Analysis

Helen Williams*
*AstraZeneca, Silk Road Business Park, Macclesfield, Cheshire, UK. SK10 2NA

EPR spectroscopy has been routinely used by AstraZeneca over the last 8 years to support the development of active pharmaceutical ingredients (APIs) and formulated products. Examples from the main application areas will be presented including photodegradation and oxidation studies of APIs and formulated products, the effect of manufacturing processes on the free radical content of excipients and the use of γ-irradiation as a method of terminal sterilisation.

The pharmaceutical regulatory authorities require stability testing to be performed for all new APIs and formulated products and all main degradation mechanisms to be well understood. EPR spectroscopy has previously been used to identify free radical intermediates during autoxidation [1] and photodegradation of numerous APIs but more recent studies designed to predict the longer term stability characteristics of an API based on short EPR experiments will be presented. These studies can be used early in the development of an API, instead of the more time consuming and resource intensive standard stability testing. Degradation studies of formulated products will also be presented including whole tablet analysis and spin trapping experiments for solution formulations.

Certain excipients used routinely in pharmaceutical formulations are known to contain [2] and also form free radicals during processing [3]. Studies have been performed to identify the process steps that result in free radical formation and those that can subsequently reduce the radical content of an excipient. This knowledge can then be used to control the free radical content of the final pharmaceutical product and so reduce the degradation of a susceptible API. This is particularly important for inhalation products where the process steps designed to deliver lactose, a commonly used excipient, with the required particle size for inhalation into lung have been shown to result in free radical formation.

Terminal sterilisation by γ-irradiation is often a preferred option when product sterility is required, due to its low cost. This process can however result in significant free radical formation in the API [3], excipients [3, 4] or even packaging of an irradiated pharmaceutical product. EPR spectroscopy has therefore been used in a number of studies to determine the effect of γ-irradiation on the free radical content of product and the stability and fate of the radicals.

[1] Williams H., Loades V., Claybourn M., Murphy D. *Anal Chem* **2006** 78(2), 604-608
[2] Williams H., Claybourn M. *Spectroscopy Europe* **2006** 18(1) 10-16
[3] Raffi J., Gelly S., Barral L., Burger F., Piccerelle P., Prinderre P., Baron M., Chamayou A., *Spectrochimica Acta Part A* **2002** 58, 131-1320
[4] Bushell J., Claybourn M., Williams H., Murphy D. *J Cont Rel* **2005** 110, 49-57

EPR/Alanine Dosimetry: Final Chapter?

M. A. Morsy

Chemistry Department, King Fahd University of Petroleum & Minerals, Dhahran 31261, Saudi Arabia.

The increasing use of radiation sources of high activity for industrial and medical applications promotes a "radiation dosimetry" field of research to accurately measure the absorbed dose in materials due to the interaction with ionizing radiation [1]. EPR-dosimeters are either inorganic such as chorales, bones, minerals, or organic such as amino acids (alanine) and sugars [2]. When alanine is exposed to ionizing radiation, stable free radicals are created, and a resolved EPR spectrum with low background appears. This dose response is highly stable, reproducible, cumulative, non-destructive, linear over 3-4 decades, and nearly independent to variations in dose rate, photon energy, radiation quality (low LET), minor temperature changes, and other ambient conditions [3].

In this study, simple design of polycrystalline alanine-in-glass (AiG) dosimeter has been used to explore different ionizing irradiation sources, including direct gamma and Linac X-ray, were used to irradiate AiG dosimeters with low-dose typical for medical therapy ranging from 0 to 20 Gy as well as high-dose for syringes' sterilization processes ranging from 5 to 50 kGy. This design avoids preparation complications such as mortaring, sieving, and/or binder addition. It also reduces several fabrication techniques of alanine dosimeters and hence allows other laboratories to conduct similar EPR measurements. An external standard reference (Mn^{2+}/MgO) was used to correct for any sensitivity variations in the spectrometer response. The dosimeter was found to be more sensitive to low radiation doses than other types of alanine dosimeters such as alanine pellets and fine powder. They exhibited a linear dose response in the examined dose ranges. This practical method and the well known characteristics of alanine dosimeters favour the polycrystalline AiG as a radiation dosimeter in medical applications, especially the conventional radiotherapy treatment. Further analysis was carried out for the "stable radical-types" of alanine.

[1] W. L. Mclaughlin, A.W. Boyd, K. Chadwick, J. C. McDonald, A. Miller, "*Dosimetry for Radiation Processing"*, Taylor & Francis, London-New York-Philadelphia, **1989**.

[2] M. Ikeya, J. Miyajima, S. Okajima. "*ESR dosimetry for Atomic Bomb Survivors Using Shell Butons, and Tooth Enamel"*, Japan J. Appl. Phys., 23, 679, **1984**.

[3] "*Standard practice for use of an alanine-EPR dosimetry system*". American Society for Testing and Materials, ISO/ASTM 51607:**2004**.

Electronic structure of large polymetallic molecules: a multi-frequency EPR approach

David Collison

School of Chemistry, The University of Manchester, Oxford Road,
Manchester M13 9PL, UK.

An understanding of magnetic exchange coupling between paramagnetic metal ions was first reported in 1952 by Bleaney and Bowers,[1] and a polymetallic, molecular ring (or wheel) of metal ions was first identified in 1985 by Timco.[2] These molecular, large (*ca.* 2 nm diameter) objects are intrinsically interesting because of their beautiful structures, huge range of variation in component parts (metal ions, co-ligands, bridging groups) and the challenge of successfully describing their electronic structure. We have concentrated upon a family of compounds with many siblings,[3] in which metal content, $\{M^{III}_{p}M^{II}_{q}\}$ (commonly p = 6-9, q = 1-2) defines the key structural facet, bridging ligands are typically carboxylates, fluorides and/or alkoxides, there is a templating ion inside the ring, and the organic components can be multi-functionalised to lead to predictable, complex architectures. Thus, these molecules have been suggested as components in one realisation of quantum information processing (QIP).

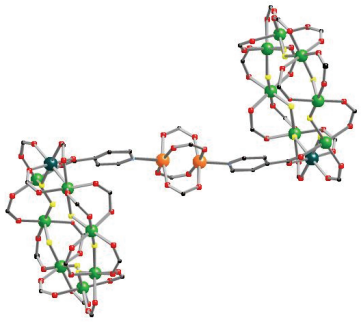


Fig. 1. Structure of a $\{Cr_7Ni\}$-$\{Ru_2\}$-$\{Cr_7Ni\}$ linked-rings molecule, **1**.

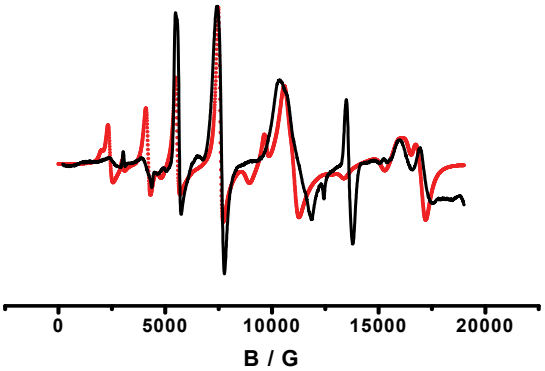


Fig. 2. Q-band EPR spectrum and simulation of **1**, at 5 K, from which $-J_{ring\text{-}Ru_2} = 0.13(1)$ cm^{-1} can be derived.

We report the development of a model[4] that describes the electronic structure of these molecular rings, *e.g.* "Cr_7Ni", which has relied principally, but not exclusively, upon multi-frequency EPR. Figures 1 and 2 show an example. The extension of this model to develop more complicated designs of the overall electronic structure with multiple rings will be presented.

[1] B. Bleaney and K.D. Bowers, Proc. Roy. Soc. A, 1952, 214, 451.
[2] G.A. Timko et al., Patent SU No. 1299116, Priority date: 1985-08-07.
[3] R.E.P. Winpenny et al., Chem. Commun., 2005, 1125.
[4] S. Piligkos et al., Chemistry - A European Journal, 2009, 15, 3152.

An EPR Investigation of non-C_2 Symmetric Cu(Salen) based Complexes

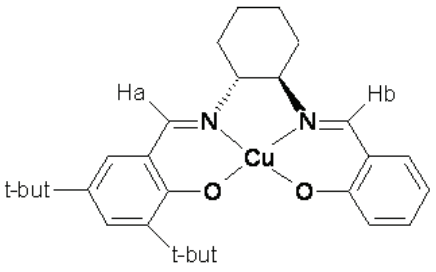
E. Carter,[#] *I. Caretti*,[*] *D. M. Murphy*,[#] *I. A. Fallis*,[#] *S. van Doorslaer**

[#]*School of Chemistry, Cardiff University, Main Building, Park Place, Cardiff, UK.*
[*]*University of Antwerp, Department of Physics, SIBAC laboratory, Universiteitsplein 1, B-2610 Wilrijk, Belgium.*

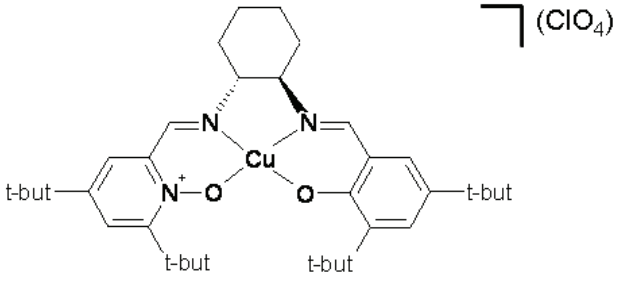
The requirement for obtaining optically pure enantiomers of small organic compounds is important in the fields of synthetic organic chemistry, pharmaceuticals and industrial catalysis. This need has resulted in a large volume of work in asymmetric catalysis, often utilising transition metal complexes of chiral Schiff base ligands. It is well known that the electronic and structural properties of the ligand play a central role in determining the yield and enantioselectivity of catalytic processes.

In the current work, we will demonstrate how a combination of EPR, ENDOR and additional HYSCORE spectroscopy can be used to structurally characterise two very different non-C_2 symmetric complexes based on the salen motif; [Cu(**1**)] and [Cu(**2**)](ClO_4). In [Cu(**1**)] we will examine the nature of the adducts formed with the chiral amine MBA (methylbenzylamine). In [Cu(**2**)] we will report how the inherent asymmetry in the ligand itself affects the spin distribution in the metal complex. We observe that polarization of the electron density at the metal centre leads to a large asymmetry of [Cu(**1**)]+MBA and [Cu(**2**)], readily observable *via* two distinct hyperfine coupling patterns originating from the non-equivalent H_a and H_b imine protons.

The implications of these results within the context of outer sphere interactions in asymmetric catalysis are considered.



[Cu(**1**)]



[Cu(**2**)]

EPR investigations on mutants of the membrane-bound [Ni-Fe]-hydrogenase from *Ralstonia eutropha*

Ch. Teutloff[*], J. Priebe [**], J. Löwenstein [*], J. Fritsch [***], B. Friedrich [***], P. Hildebrandt [**], O. Lenz [***], I. Zebger [**], F. Lendzian [**], R. Bittl [*]

[*]Institut für Experimentalphysik, Freie Universität Berlin, Arnimallee 14, 14195 Berlin , Germany.
[**]Max-Volmer-Labor, Technische Universität Berlin, Straße des 17. Juni 135, 10623 Berlin, Germany.
[***]Institute für Biologie/Microbiologie, Humboldt-Universität zu Berlin, Chausseestrasse 117, 10115 Berlin, Germany

Understanding the catalytic mechanism of reversible hydrogen cleavage into protons and electrons by hydrogenases is an important step towards the application of their catalytic centres as building blocks for H_2 production. Particularly interesting is the class of oxygen-tolerant [Ni-Fe]-hydrogenases as they are able to metabolize H_2 at ambient oxygen levels. The origin of this remarkable O_2-tolerance is still under vivid discussion. Here we focus on the membrane-bound hydrogenase (MBH) from *Ralstonia* (*R.*) *eutropha* H16.

Since many intermediate redox-states of hydrogenases are paramagnetic, EPR spectroscopy is the method of choice for their investigation. EPR studies have shown that the electronic structure of the catalytic [NiFe]-centre in MBH from *R. eutropha* is virtually identical to standard hydrogenases [1]. However, the enzyme exhibits in its (resting) Ni-B state a magnetic coupling between the [NiFe]-centre and two paramagnetic species, a distinct feature not found in standard hydrogenases.

Here EPR investigations are presented on *R. eutropha* deletion mutants of genes presumably involved in the maturation of the iron-sulfur (FeS) clusters in the electron-transfer chain of MBH located in the small subunit of the enzyme. Additionally point mutants were investigated which addressed amino acids probably involved in unique ligation of the FeS clusters. Attention is turned to the unique electronic properties (i.e. magnetic coupling to the [NiFe]-centre) of the iron-sulfur clusters, which might be related to redox features responsible for O_2- tolerance.

This research is supported by the DFG within cluster of excellence “Unicat”.

[1] M. Saggu, C. Teutloff, M. Ludwig *et al.*; PCCP, 12(9),2139-2148, **2010**.

HYSCORE and ENDOR spectroscopy to determine the g-matrix orientation of the reduced [2Fe-2S] cluster in palustrisredoxin

Joseph Abdalla, Alice Bowen, Janet Lovett, Christiane R Timmel, Stephen Bell, Nicki Hoskins, Luet Lok Wong, Jeffrey Harmer (jeffrey.harmer@chem.ox.ac.uk)

Centre for Advanced Electron Spin Resonance and Inorganic Chemistry Laboratory, Department of Chemistry, South Parks Road, Oxford, OX1 3QR, U. K.

Electron transfer proteins containing FeS clusters play a critical role in many catalytic reactions carried out at the active sites of proteins. We have been studying the heme dependent cytochrome P450 enzymes from *Rhodopseudomonas palustris* [1] which is responsible for catalytic hydroxylation. In this systems electrons are delivered to the heme group via a two protein electron transport chain; a flavoprotein reductase reduces a ferredoxin with a [2Fe-2S] cluster that in turn transfers electrons to the substrate-bound Fe^{III} heme. We are currently determining the structure of the complex formed between ferredoxin reductase (containing a $NO^{\bullet}$ spin) and the ferredoxin (with a reduced [2Fe-2S] cluster) via DEER spectroscopy and consequently the orientation of the reduced [2Fe-2S]-cluster g-matrix is required in the analysis.

Previously Kappl *et al.* studied the reduced [2Fe-2S] cluster in adrenodoxin using ^{1}H X-band CW ENDOR to determine the g-matrix orientation and spin projection factors for the anti-ferromagnetically coupled Fe^{3+} and Fe^{2+} ions.[2] We have adapted their approach to our ferredoxin system and combined W-band ^{1}H Davies ENDOR with X-band HYSCORE to obtain a detailed description of the reduced, S=½, [2Fe-2S] cluster. Our g-matrix orientation is in excellent agreement with the results of Kappl *et al.*, but our spin projection factors are significantly larger and we believe more accurate as a result of the combination of ENDOR and HYSCORE spectroscopy.

[1 F. Xu, S. G. Bell, Z. Rao and L.-L. Wong, *J. Mol. Biol.*, 2008, **383**, 561-574.

[2] R. Kappl, M. Ebelshäuser, F. Hannemann, R. Bernhardt, J. Hüttermann, *Appl. Magn. Reson.*, 2006, **30**, 427-459.

Origin of Light-Induced Spin-Correlated Radical Pairs in Cryptochromes and Related Blue-Light Active Proteins

Till Biskup[*,†], Erik Schleicher[*], Asako Okafuji[*], Anthony R. Marino[**], Kenichi Hitomi[***], Elizabeth D. Getzoff[***], James R. Norris[**], Stefan Weber[*]

[*]Institute of Physical Chemistry, Albert-Ludwigs-Universität Freiburg, Freiburg, Germany.
[**]Department of Chemistry, University of Chicago, Chicago, U.S.A.
[***]Skaggs Institute for Chemical Biology, Scripps Research Institute, La Jolla, U.S.A.
[†]Present address: Physical and Theoretical Chemistry Lab., University of Oxford, Oxford, UK.

Blue-light excitation of cryptochromes (Crys) and homologs uniformly triggers electron transfer (ET) from the protein surface to a flavin-adenine dinucleotide (FAD) cofactor [1]. A cascade of three conserved tryptophan (Trp) residues has been considered to be critically involved in this photoreaction. Starting from fully oxidized FAD, light-induced ET via the Trp triad generates a series of short-lived spin-correlated radical pairs (RPs) comprising of an $FAD^{\bullet}$ and a $Trp^{\bullet}$. Coupled doublet-pair species of this type have been proposed as the basis, e.g., of a biological magnetic compass in migratory birds, and were found critical for some Cry functions *in vivo* (see, e.g., [2]). In this contribution, Cry-like proteins derived from *Xenopus laevis* and *Synechocystis* sp. PCC6803 have been examined as paradigm systems. Light-generated RP states $FAD^{\bullet}\cdots Trp^{\bullet}$ have been characterized in detail by time-resolved EPR (TREPR) at various microwave frequencies. Different RP precursor states – singlet versus triplet – have been considered in spectral simulations of the experimental spin-polarized TREPR signals. Conclusively, we present evidence for a singlet-state precursor of $FAD^{\bullet}\cdots Trp^{\bullet}$ RP generation because at the different magnetic fields, where the RPs were studied by TREPR, net-zero spin polarization was observed. Neither a spin-polarized triplet precursor nor a triplet at thermal equilibrium can explain such a pattern. It turns out that a multi-frequency TREPR approach is essential to draw conclusions on the nature of the precursor electronic states in light-induced spin-correlated RP formations [3].

Very recently, light-induced RP species have also been detected in other flavin-based photoreceptors with different function [4]. The origin of the RP species is, however, dissimilar to those in Crys. Apparently, flavin chromophores can provide quite flexible photochemistry, which is optimized with respect to protein function.

[1] T. Biskup, E. Schleicher, A. Okafuji, G. Link, K. Hitomi, E.D. Getzoff, S. Weber, *Angewandte Chemie International Edition* 48, **2009**, 404–407.

[2] C.T. Rodgers, P.J. Hore, *Proceedings of the National Academy of Sciences of the United States of America* 106, **2009**, 353–360.

[3] S. Weber, T. Biskup, A. Okafuji, A.R. Marino, T. Berthold, G. Link, K. Hitomi, E.D. Getzoff, E. Schleicher, J.R. Norris, *Journal of Physical Chemistry B* 114, **2010**, 14745–14754.

[4] S. Weber, C. Schroeder, S. Kacprzak, T. Mathes, R.M. Kowalczyk, L.-O. Essen, P. Hegemann, E. Schleicher, R. Bittl, *Photochemistry and Photobiology* **2011**, in press.

Tryptophan radicals

Stefan Stoll[†], Hannah S. Shafaat[‡], Jurek Krzystek[§], Andrew Ozarowski[§], Michael J. Tauber[‡], Judy E. Kim[‡], R. David Britt[†]

[†] Department of Chemistry, University of California Davis, Davis, California, U.S.A.
[‡] Department of Chemistry and Biochemistry, University of California San Diego, La Jolla, California, U.S.A.
[§] National High Magnetic Field Laboratory, Tallahassee, Florida, U.S.A.

Tryptophan (Trp) radicals serve as relays in multi-step electron transfer through proteins and as oxidants in biotechnologically interesting enzymes that can oxidize high-reduction-potential organic substrates such as lignin. Despite their relevance, little is known about the electronic and magnetic structure of these Trp radicals.

We use multi-technique EPR to elucidate the structure of two model Trp radicals, photogenerated in the electron-transfer protein azurin. One radical is located in a solvent-exposed region close to the protein surface, and the other in a hydrophobic pocket in the core of the protein. Q-band ^{1}H ENDOR gives spin density distributions and side chain conformations. H/D exchange probes the presence of hydrogen bonds. CW EPR at uniquely high field and frequency (25 T, 700 GHz) is used to completely resolve the very narrow g tensors (g_{max}-g_{min} < 0.0015) and determine the g values using endohedral atomic hydrogen as a field standard.

Both Trp radicals are found to be neutral. The surface radical is hydrogen bonded to a water molecule, whereas the core radical is not involved in hydrogen bonds. The g tensors are significantly different and reflect the difference in electronic environment and H-bonding. For both radicals, side chain conformational distributions were found and quantified.

Alzheimer amyloid peptide aggregation studied by SL-EPR

M.H. Shabestari[a], I. Sepkhanova[a], M. Drescher[a], N.J. Meeuwenoord[b], R.W.A.L. Limpens[c], R.I. Koning[c], D.V. Filippov[b], M. Huber[a]

[a]Department of Molecular Physics, Huygens Laboratory, Leiden University, P.O. Box 9504, 2300RA Leiden, The Netherlands
[b]Leiden Institue of Chemistry, Leiden University, Leiden, The Netherlands
[c]Leiden University Medical Center, Department of Molecular Cell Biology, Section Electron Microscopy, Leiden, The Netherlands

The aggregation of the β-Amyloid (Aβ) peptide into fibrils and plaques is the chief indicator of Alzheimer's disease. [1] Specific interest in oligomers stems from the suggestion that small, oligomeric aggregates and protofibrils, rather than fully formed fibrils could be responsible for the toxicity of the Aβ-peptide.

We investigate the potential of EPR to detect early stages of the aggregation of the Aβ-peptide. We have labeled the 40 residue Aβ peptide variant containing an N-terminal cystein (cys-Aβ) with the MTS spin label (SL- Aβ).

Continuous wave, 9 GHz EPR reveals three fractions of different spin-label mobility. One attributed to monomeric Aβ, one to a multimer (8 to 15 monomers), and the last one to larger aggregates or fibrils. (Fig. 1) The approach allows detection of oligomers on the timescale of aggregation. [2]

The future direction in this research is to study the effect of different conditions on the process of fibrilization and on the time scale of aggregation.

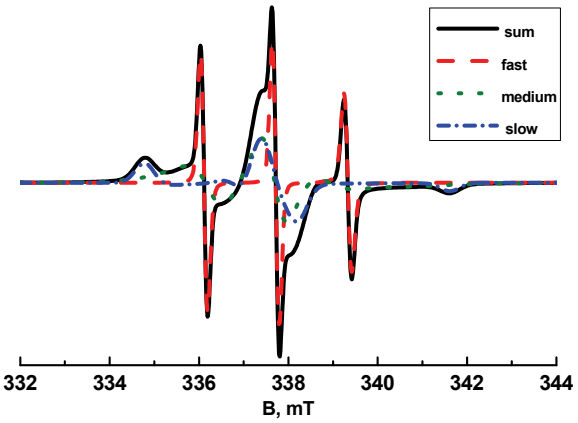


Fig. 1 simulation of the SL-Aβ spectrum with three components: dashed line fast, dotted line medium, short dashed line slow.

[1] Hardy J & Selkoe DJ. Science, 297, 353-356. (2002)
[2] Sepkhanova, I., et al. Applied magnetic resonance,36, 209-222. (2009)

ESR-spectroscopy in ionic liquids: Investigations on the dynamics and kinetics of organic radicals

G. Grampp*, B. Mladenova and D. Kattnig
Institute of Physical and Theoretical Chemistry, Graz University of Technology,
Stremayrgasse 9, A-8010 Graz (Austria). e-mail: grampp@TUGraz.at

Ionic liquids are molten salts of mainly organic cations and inorganic or organic anions, liquid at room temperature [1]. They are of interest, not only from scientific point of view but also for chemical industrial productions. Environmental friendly industrial productions based on so-called "Green Chemistry" principles mainly use ionic liquids as solvent to avoid classical toxic organic solvents. The ionic liquid structures, consisting of cations and anions, are completely different from classical organic bulk solvents. Ionic liquids are used for example in electrochemistry with great success since the electrochemical window is larger than 6 volts.

But only a few reports exist on ESR-spectroscopic investigations using ionic liquids as solvents. We would like to report on ESR-measurements using the ionic liquids: 1-butyl-3-methylimidazolium hexafluorophosphate ($bmim^+PF_6^-$), 1-butyl-3-imidazolium fluoroborate ($bmim^+BF_4^-$) and 1-ethyl-imidazolium ethylsulfate ($emim^+O_3SOEt^-$).

a) Electron-self exchange

We report on the electron-self exchange rates of various organic redox-couples: MV^+/MV^{++} (methylviologene), EV^+/EV^{++} (ethylviologene) $TCNE^-$/TCNE (tetracyanoethylene), $TCNQ^-$/TCNQ (tetracyanoquinionedimethane) $TMPPD^+$/TMPPD (N,N,N',N'-tetramethyl-p-phenylenediamine) and TEMPO/$TEMPO^+$. From ESR-linebroadening experiments rate constants corrected for diffusion vary between 8.2 $x10^7$ $M^{-1}s^{-1}$ and $1.2x10^9$ $M^{-1}s^{-1}$, depending on the ionic liquid used [2]. The activation energies range from $\Delta G^* = 27.4 - 42.1$ kJ/mol. These results will be compared with measurements obtained in different common classical organic solvents [3]. The solvent dependent outer-sphere reorganization energy is discussed in the sense of Marcus-Theory. This theory is not applicable to ionic liquids.

b) Rotational correlation times τ_{rot}

Using the high viscosity of ionic liquids, the rotational correlation times, τ_{rot}, of uncharged TEMPO derivatives and the charged Fremy's salt are measured in $bmim^+PF_6^-$ and $emim^+O_3SOEt$. τ_{rot}(TEMPO, $bmim^+PF_6^-$) = $4.0x10^{-10}$ s^{-1} and τ_{rot}(Fremy, $bmim^+PF_6^-$) = $4.3x10^{-10}s^{-1}$ are obtained, both at T=300K. These results are similar to published data [4,5,6]. Temperature dependent measurements (298K$\leq$ T$\leq$450K) reflect the viscosity dependence of τ_{rot}. We also report on **pressure dependent** ESR-measurements of τ_{rot} up to 800 bar.
The Debye-Stokes-Einstein relation is always fulfilled.

[1] T. Welton, Chem.Rev. **1999**,*99*,2071.
[2] G. Grampp, D.Kattnig and B. Mladenova, *Spectrochim.Acta A* **2006**, *63*, 821.
[3] G. Grampp, B. Mladenova, D. Kattnig and S. Landgraf, *Appl.Magn. Reson.* **2006**, *30*, 145.
[4] R. G. Evans, A. J. Wain, C. Hardarce and R. G. Compton, *ChemPhysChem*. **2005,** *6*, 1035.
[5] A. Kawai, T. Hidemori and K. Shibuya, *Chem.Lett*. **2004,** *33*, 1464.
[6] A. Stoesser, W. Herrmann, A. Zahl, V. Strehmel and A. Laschewsky, *ChemPhysChem.* **2006**, *7,* 1106.
[7] A. T. Bullock, and C. B. Howard, *J. Chem. Soc. Faraday Trans. 1*,**1980**, *76,* 1296-1300.
Financial support from FWF-project: I 190-N 17 is gratefully acknowledged.

Pulsed ENDOR Spectroscopy of the S_2-State Multiline Signal of Photosystem II

Susanne Pudollek[*], Christian Teutloff[*], Matthias Broser[**], Julia Hellmich[**], Athina Zouni[**], Robert Bittl[*]

[*]Fachbereich Physik, Freie Universität Berlin.
[**]Max-Volmer-Laboratorium, Technische Universität Berlin.

The oxygen evolving complex (OEC) in photosystem II (PSII) is the catalytic core where water splitting and oxygen evolution takes place. Water oxidation occurs during the five steps ($S_{0–4}$) of the redox-cycle (Kok-cycle) [1]. The OEC consists of four Mn ions and one Ca ion [2]. Due to radiation damage [3] and resolution limitations of the crystal structure [4] the bridging ligands between the metal ions and the protein ligands of the cluster have not been unequivocally identified yet. However, the knowledge of the electronic and geometric structure of the OEC as well as its ligand surrounding is the foundation for a mechanistic understanding of the OEC catalysed water splitting.

EPR spectroscopy is well suited to gain information on the electronic structure and subsequently on the geometry of radicals and paramagnetic centers. ^{55}Mn-ENDOR spectroscopy can directly provide information on the hyperfine couplings of the OEC.

First, ^{55}Mn-ENDOR spectroscopy was applied to the S_2-state of the OEC in PSII single crystals. Previous analysis allowed a tentative assignment of the Mn ion in the OEC carrying the largest hyperfine coupling, i.e. likely the oxidation state III, to two out of the four Mn positions in the structure. Here, we present a more comprehensive analysis of the data leading to a preliminary assignment of hyperfine couplings to Mn ions in the structure.

A second question concerns the Ca ion in the OEC. Ca is a functional important part of the OEC as without Ca activity is lost. The only known functional substitute for Ca^{2+} is Sr^{2+}. To learn more about the role of Ca^{2+} we investigated the effects of Sr-substitution for Ca on the electronic structure of the OEC in the S_2-state by ^{55}Mn-ENDOR.

In addition ^{1}H-/^{2}H-ENDOR spectroscopy was used to study the exchangeable hydrogens in the vicinity of the OEC. Species dependent differences of hyperfine couplings to previous studies [5] will be discussed.

[1] B. Kok *et al.*, *Photochem. Photobiol.*, 11, 457–475, **1970**.
[2] J.P. McEvoy *et al.*, *Chem. Rev.*, 106, 4455–4483, **2006**.
[3] J. Yano *et al.*, *Proc. Natl. Acad. Sci. USA*, 102, 12047–12052, **2005**.
[4] A. Guskov *et al.*, *Nat. Struct. Mol. Biol.*, 16, 334–342, **2009**.
[5] C.P. Aznar *et al.*, *Phil. Trans. R. Soc. B*, 357, 1359–1366, **2002**.

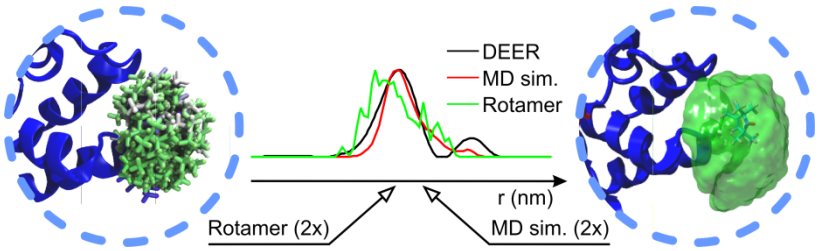
Comparative DEER- & FRET distance determination in simulation & experiment

Daniel Klose[*], Dina Grohmann[$], Johann P. Klare[*], Finn Werner[$], Christopher W.M. Kay[$], Heinz-Jürgen Steinhoff[*]
[*]*University of Osnabrück, Barbarastr. 7, 49076 Osnabrück, Germany,*
[$]*University College London, Gower Street, London WC1E 6BT, UK*
dklose@uos.de

Structural elucidation based on inter label distance determination by DEER or FRET faces a resolution limit mainly determined by the number of available constraints and by the deviation of the label centers from the protein backbone. Since experimental inter label distances (or distributions) cannot directly be translated to constraints in a structural model, different simulation techniques are used to provide orientation distributions of labeled side chains. Achieving correctly simulated distributions is essential if precise constraints are to be derived.

Here we present results on this problem using a model system, the subunits F and E from the archaeal RNA polymerase[1], which form a stable heterodimer $(F/E)_2$.

In this study we focus on a comparative evaluation of experimental and simulated distances, or distributions for DEER and FRET, respectively. Cysteine mutants within F/E were spinlabeled for DEER- or derivatized with fluorescence labels for FRET experiments. Accordingly, simulated orientation distributions of the labeled side chains are calculated using conformational sampling[3] by molecular dynamics- (MD) as well as by Monte Carlo (MC) simulations and, for the spin labels, by a rotamer library based approach[4]. This versatile approach allows not only for comparison of experimentally determined inter-label distances (or distributions) to the simulated orientation distributions for both EPR- & FRET labels, but additionally, it allows for cross validation of the different simulation techniques and protocols. As exemplified in the figure, for spin labels the comparison shows reasonable agreement, especially in terms of the mean distances, while for FRET labels simulations prove to be possible yet more challenging.



This comparative study is aimed at developing the simulation methodology in order to increase the efficacy of structural modeling of proteins.

References

1. Werner, F. (2008) *Trends Microbiol.* **16**, 247-250
2. Todone, F., Brick P., Werner F., Weinzierl R.O., Onesti S. (2001) *Mol.Cell* **8**, 1137-1143
3. Beier, C. and Steinhoff, H.-J. (2006) *Biophys. J.* **91**, 2647-2664
4. Polyhach, Y., Bordignon E., Jeschke, G. (2011) *Phys. Chem. Chem. Phys.***13**, 2356-2366

Ghost distances in double electron-electron resonance of multiple-spin systems

Tona von Hagens, Yevhen Polyhach, Gunnar Jeschke

Laboratory of Physical Chemistry, ETH Zurich.

The Double Electron-Electron Resonance (DEER) experiment is an important method in structure-function studies of macromolecules, due to its ability to provide pair distances in the range of 1.5 - 8.0 nm. The presence of more than two spins within a biomacromolecule is not a rare event and commonly the case if spin-labelled oligomers are studied [1]. More than two dipolar coupled spins manifest in an increased total modulation depth and in sum and difference frequency contributions in the DEER form factor. This leads to line broadening in frequency domain and eventually to additional peaks in the distance distribution. These “ghost peaks" do not correspond to the real interspin distances of the system and may hence lead to erroneous data analysis if their origin is not taken into account.

The geometry of the particular spin system determines the position of the higher-order contribution peaks in the distance distribution. However, the extent and prominence of these peaks strongly depend on the inversion efficiency of the system that scales with the pump pulse power of the spectrometer. An accurate knowledge of spin pair geometry and inversion efficiency is necessary to estimate whether higher-order contributions can be neglected in first approximation or have to be taken into account.

Approaches to separate the pair contribution from higher-order contributions in order to obtain true interspin distances have been developed and tested on simulated DEER traces and model samples. The elimination of higher-order contributions with these approaches leads to a considerably improved resolution of the distance distribution and prevents erroneous interpretation of the distribution.

[1] Jeschke, G.; Sajid, M.; Schulte, M.; Godt, A. Phys. Chem. Chem. Phys. 2009, 11, 6580-6591.

Exploring the Binding Site of Ubiquinone in Complex I by EPR/DEER

Müge Aksoyoglu*, Udo Glessner**, Thomas Spatzal**, Erik Schleicher*, Stefan Weber*

* Department of Physical Chemistry, Albert-Ludwigs-Universität Freiburg, Germany
** Department of Biohemistry, Albert-Ludwigs-Universität Freiburg, Germany

The proton pumping NADH:ubiquinone oxidoreductase, the respiratory complex I, couples electron transfer from NADH to ubiquinone with the translocation of protons across the membrane [1]. Ubiquinone, which binds to complex I, is a key component of the electron-transport chain because it delivers electrons from complex I to complex III. Recently, the three-dimensional structure of the entire complex I from *Thermus thermophilius* was determined [2]. However, the structure does not show the presence of bound quinone but a large cavity at the end of the peripheral arm was proposed as binding site. In this study, site-directed spin labelling (SDSL) in combination with EPR/DEER spectroscopy is used to localize the ubiquinone binding site in complex I on a molecular level. Various positions in the protein as well as decyl-ubiquinone (Q_{10}) were spin labelled with MTSL to measure distances between the two labels via cw-EPR and DEER experiments. We present some of our results considering the binding site of ubiquinone.

Moreover, we present preliminary data on the FeMo cofactor from nitrogenase, which reduces the dinitrogen triple bond under atmospheric pressure and temperature, thereby forming ammonia in a reaction coupled to the hydrolysis of ATP [3]. Various EPR and HYSCORE methods were used to investigate the FeMo cofactor, which is not yet fully understood in terms of its composition.

[1] Pohl, T., Spatzal, T., Aksoyoglu, M., Schleicher, E., Rostas, A.M., Lay, H., Glessner, U., Boudon, C., Hellwig, P., Weber, S., Friedrich, T., Spin labelling of the *Escherichia coli* NADH ubiquinone oxidoreductase (complex I). *Biochim. Biophys. Acta* 2010, **1797**, 1894–1900.
[2] Efremov, R.G., Baradaran, R., Sazanov, L.A., The architecture of respiratory complex I. *Nature* 2010, **465**, 441–447.
[3] Lee, H., Benton, P., Laryukhin, M., Igarashi, R., Dean, D.R., Seefeldt, L.C., Hoffmann, B.M., The interstitial atom of the nitrogenase FeMo-cofactor: ENDOR and ESEEM show it is not exchangeable nitrogen. *J. Am. Chem. Soc.* 2003, **125**, 5604–5605.

EPR study in magnetoresistive $GdBaCo_2O_{5+\delta}$

Cesare Oliva*, Mattia Allieta*, Serena Cappelli*, Marco Scavini*, Alessandro Lascialfari**

*DCFE, University of Milan and CNR-ISTM.
**Department of Molecular Sciences Applied to Biosystems, University of Milano, and S3-CNR-INFM, Modena (Italy).

The magneto-resistance (MR) property in oxides containing perovskite building blocks is one of the central issues in condensed matter physics both for scientific and technological interest. In order to clarify the MR mechanisms recently the layered cobalt oxides $GdBaCo_2O_{5+\delta}$ have been widely considered as a model system [1].

These compounds show complex transport and magnetic properties as a function of δ and temperature [1]. In particular for δ=0.5-0.65 and T>300K the system is paramagnetic and displays metal-insulator (MI) transition across T_{MI}~340K. For T<300K, magnetization measurements show the occurrence of a PM to ferromagnetic (FM) transition followed by a FM to antiferromagnetic (AF) transition.

Many efforts have been made to explain the origin of the MI transition in this compounds and recently *ab initio* calculations indicated that this transition must be accompanied by a spin state transition from High Spin (HS) (S = 2) to Intermediate Spin (IS) (S=1) state for Co^{3+} ions in octahedral environment, while those in square-pyramidal environment would be stable in their IS (S=1) state [2].

We present, to the best of our knowledge, the first EPR investigation on powdered $GdBaCo_2O_{5+\delta}$ (δ=0.54(1), 0.57(1), 0.63(1)) samples, carried out in the 120K<T< 450K range.
The EPR features were underlining all the above significant transitions in 120<T<450 K range. For data collected at T>300K we performed an accurate line-shape analysis. The trend of the line-width changed markedly at temperature values close to T_{MI}. Our results, supported by SQUID analysis at T<300K carried out on the same samples, contribute to enlighten the mechanism of the magnetic and metal-insulator transitions occurring in this system.

[1] A. A. Taskin, A. N. Lavrov, Y. Ando, *Phys Rev* **B71** (2005) 134414.
[2] V.Pardo, D.Baldomir, *Phys. Rev.* **B73**, (2006) 165117

NO as a magnetic probe to investigate inhibition of Ni-Fe hydrogenase

Emilien Etienne[*], Bénédicte Burlat[*], Pierre Ceccaldi[*], Pierre-Pol Liebgott[*], Sébastien Dementin[*], Marc Rousset[*], Christophe Léger[*], Bruno Guigliarelli[*]

[*] Laboratoire de Bioénergétique et Ingénierie des Protéines, BIP-UPR9036, CNRS and Aix-Marseille University, 31 Chemin J. Aiguier 13402 Marseille cedex 20 France.

Hydrogenases are enzymes that reversibly catalyse the oxidation of molecular hydrogen into protons and electrons in microorganisms. They are classified into three groups depending on the metal content of their active site: [Fe], [FeFe] and [NiFe] hydrogenases. These enzyme systems have been extensively studied for many years by numerous techniques and approaches and all parts of their mechanism have been investigated, from the substrate activation at the active site to electron transfer through the iron-sulfur clusters, and protons transfer. A special attention was devoted to understand the inhibition processes undergone by these enzymes in light of their potential applications for biohydrogen production and biobattery development [1-4].

In standard Ni-Fe hydrogenases, like that from *Desulfovibrio fructosovorans*, the crystal structure of the enzyme has revealed the existence of hydrophobic channels that connect the buried active site to the surface of the protein, thus enabling diffusion of H_2 or of gaseous inhibitors of the enzyme as O_2, CO or NO. Whereas O_2-inhibition of hydrogenases was largely studied in the context of their potential applications in biotechnological devices [1,3,4], the mechanism of NO-inhibition remains less well-characterized [5,6].

We have investigated the interaction of *D. fructosovorans* hydrogenase with NO by CW EPR spectroscopy. This technique enables to follow selectively the NO effects on both the Ni-Fe active site and the iron-sulfur clusters of the enzyme. While some alterations of the metal centers by NO appear to be reversible, the iron-sulfur clusters exhibit different sensitivity to irreversible damages which suggests dynamic conformation fluctuations of the enzyme.

[1] Leroux F., *Proc. Natl. Acad. Sci. USA,* **2008**, 32, 11118-11193.
[2] Baffert C., *J. Am. Chem. Soc*, **2011**, ASAP.
[3] Liebgott P.-P., *J. Am. Chem. Soc*, **2011**, 133 (4), 986-997.
[4] Liebgott P.-P, *Nat. Chem. Biol.*, **2010**, 6(1), 63-70.
[5] Hyman M. R., *Biochem. J*, **1988**, 254 (2), 469-475.
[6] Hyman M. R., *Biochim. Biophys. Acta*, **1991**, 1076 (2), 165-172.

EPR, MS, XRD and TEM study of $Mn_xZn_{1-x}Fe_2O_4$ nanoparticles

Z. Klencsár, L. Korecz, Gy. Tolnai, E. Kuzmann, I. Sajó, P. Németh

Chemical Research Center, Hungarian Academy of Sciences, Pusztaszeri út 59-67., Budapest, 1025, Hungary

The $ZnFe_2O_4$ spinel and related compounds attract considerable scientific attention among others on the ground of the peculiar interdependences they display between the particle size, the Néel temperature, and the applied preparation technique [1]. Decreasing particle size was reported to lead to an increase in the magnetic ordering temperature, which effect is thought to be rooted in a change of the spinel's inversion parameter (i.e. the concentration of Fe ions on tetrahedral sites) with particle size. In the case of powders made of the mixed ferrite $Mn_xZn_{1-x}Fe_2O_4$ the particle size, the inversion parameter and the associated bulk magnetic properties have been reported to depend also on the applied Mn concentration [2].

We have prepared $Mn_xZn_{1-x}Fe_2O_4$ ($x = 0.1 \ldots 0.9$) nanoparticles via the sol-gel technique, and investigated the resulting powders by EPR spectroscopy, ^{57}Fe Mössbauer spectroscopy, XRD and TEM measurements in order to explore the possible interdependences between composition, cation distribution (inversion parameter), particle size and the magnetic properties of these materials.

The particle size of the prepared powders was found to be in the range of ~ 3-20 nm (Figure 1c,d). Mössbauer spectra indicated the presence of collective magnetic excitations and superparamagnetic relaxation in accordance with the small particle size (Figure 1b). EPR measurements revealed broadened resonance spectra (Figure 1a) with temperature- and Mn-concentration dependent line width and shape. The observed tendencies will be discussed and the results will be interpreted by taking into account the possible dependence of the inversion parameter on the applied Mn concentration.

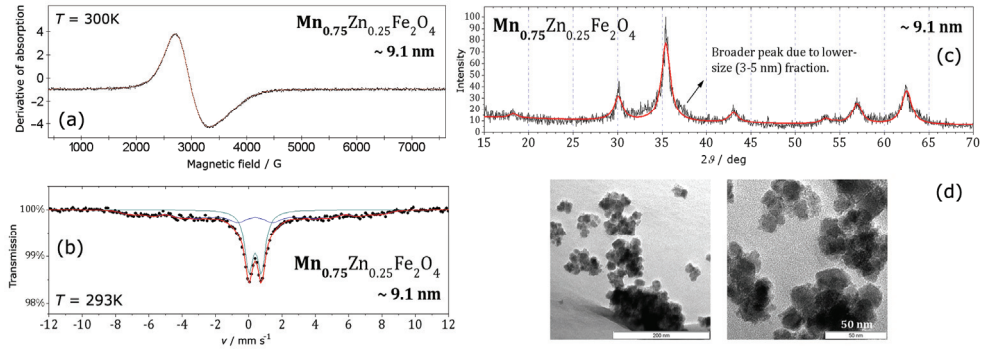


Figure 1. (a) X-band EPR spectrum (at 300K), (b) ^{57}Fe Mössbauer spectrum (at 293K), (c) X-ray diffractogram and (d) TEM image of $Mn_{0.75}Zn_{0.25}Fe_2O_4$ nanoparticles.

[1] F.S. Li, L. Wang, J.B. Wang, Q.G. Zhou, X.Z. Zhou, H.P. Kunkel, G. Williams: *J. Magn. Magn. Mater.* **268** (2004) 332–339.
[2] J. Wang, C. Zeng, Z. Peng, Q. Chen: *Physica B* **349** (2004) 124–128.

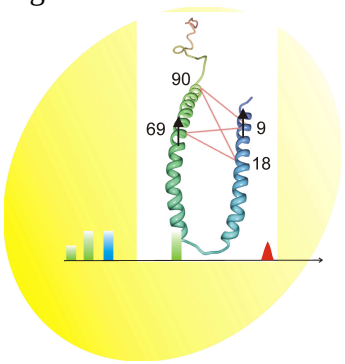
α-Synuclein, a horseshoe of (bad) luck?

M. Robotta[c], P. Braun[c], B.D. van Rooijen[b], G. Veldhuis[b], S. Milikisyants[a], V. Subramaniam[b], M. Drescher[c], M. Huber[a]

[a]Department of Molecular Physics, Leiden University, Postbox 9504, 2300 RA Leiden, The Netherlands [b]Nanobiophysics, MESA+University of Twente (The Netherlands) [c]Department of Chemistry, University of Konstanz (Germany)

Electron paramagnetic resonance (EPR) is rapidly gaining ground for structure determination in biological systems. The potential to measure nm distances between relatively small labels has been employed even to determine structural features of proteins by triangulation [1].

Fig.1



For other proteins, such as the recently discovered class of intrinsically disordered proteins, even simple questions about the structure can be a challenge. These proteins adapt to their environment, can interact with many different proteins, and, flexibility is required for their function. One representative is the protein α-Synuclein (αS)[2-5]. It was first discovered in the context of Parkinson's disease, a neurodegenerative disease that is accompanied by a shaking paralysis. αS is the main component of Lewy bodies, the brain deposits that accompany Parkinson's disease. Apparently, αS interacts with the membranes of synaptic vesicles, but the mode of interaction is still unclear. *In vitro* studies showed that αS binds to membranes adopting an α-helical structure, but the specific arrangement of the helix on the membrane was not known. Pulsed double electron-electron spin resonance (DEER or PELDOR) on spin-labelled variants of αS casts light on this question [2,4,5].

The protein can bend into a horseshoe shape (Fig. 1) [2]. On small vesicles its interaction with the membrane seems to be so strong that it can partially break up the membrane, and under these conditions, the protein forms well defined aggregates, in which two horseshoes come together in an entangled form [4]. On larger vesicles, this form still exists, but it is less stable and EPR reveals that the horseshoe and the extended forms occur side by side [5]. We speculate that the coexsistence derives from the energetic proximity of these conformations and that it is a subtle function of the environment.

References

[1] . Hilger, D et al. *Biophysical Journal* **2007**, *93*, 3675, Bhatnagar, J et al. *Two-Component Signaling Systems, Pt B* **2007**, *423*, 117.

[2] . Drescher, M.; et al. *J.Am.Chem.Soc.* **2008**, *130*, 7796-779

[3] Drescher, M.; et al. *Chembiochem* **2008**, *9*, 2411-24

[4] . Drescher, M .; et al. *J.Am.Chem.Soc.* **2010**, *132*, 4080-4081.

[5] Robotta, M.; et al. *ChemPhysChem* **2011**, *12*, 267-269.

Transmembrane gating in the type II ABC importer BtuCD-F during nucleotide cycle

Benesh Joseph*, Gunnar Jeschke[*], Kaspar P Locher[**], Birke A Goetz[**], & Enrica Bordignon[*]

[*]Laboratory for Physical Chemistry, ETH Zurich, Wolfgang-Pauli-Strasse 10, 8093 Zurich, Switzerland.

[**] Institute of Molecular Biology and Biophysics, ETH Zürich, HPK D17, Schafmattstrasse 20, CH-8093 Zürich.

ATP-binding cassette (ABC) transporters are ubiquitous integral membrane proteins that translocate substrates across cell membranes. They have a conserved architecture of two transmembrane domains bound to two cytosolic nucleotide binding domains (NBDs). The alternating-access of their transmembrane domains towards the inner and outer leaflet of the membrane powered by the closure and re-opening of the NBDs is proposed to drive the translocation events. Based on the similarity of transmembrane folds, ABC importers are classified as type I and type II [1]. Whether the structural diversity implies a different transport mechanism is still unclear. The vitamin B_{12} transporter BtuCD from *E. coli* is a type II ABC importer. BtuCD was crystallized in the presence and absence of the substrate binding protein BtuF [2, 3]. However, the absence of nucleotides in the two structures makes it impossible to delineate the sequence and the mechanism of the conformational switch during the transport cycle. We present here a detailed study of the gating mechanism of BtuCD-F during nucleotide cycle elucidated by cw and pulse EPR techniques. The transmembrane domains of the BtuCD-F complex undergo conformational changes in line with a 'two-state' alternating-access model. However, binding of ATP drives the transmembrane domains to an inward-facing conformation, in contrast to the better characterized type I importers specific for maltose, molybdate or methionine. In the presence of ATP, an excess of vitamin B_{12} promotes the re-opening of the gates towards the periplasm and the dissociation of BtuF from the transporter. The EPR data allow to model for a productive vitamin B_{12} transport cycle by BtuCD-F [4].

[1] Locher, K.P. Review. Structure and mechanism of ATP-binding cassette transporters. *Philos. Trans. R. Soc. Lond., B, Biol. Sci.* **364**, 239-245 (2009).
[2] Locher, K.P., Lee, A.T. & Rees, D.C. The E-coli BtuCD structure: A framework for ABC transporter architecture and mechanism. *Science* **296**, 1091-1098 (2002).
[3] Hvorup, R.N. et al. Asymmetry in the structure of the ABC transporter-binding protein complex BtuCD-BtuF. *Science* **317**, 1387-1390 (2007).
[4] Joseph, B., Jeschke, G., Locher, K.P., Goetz, B.A. & Bordignon, E. Transmembrane gate movements in the type II ABC importer BtuCD-F during nucleotide cycle. (*Submitted*)

Distance Measurements by DEER in Gd^{3+} – Nitroxide Radical Spin Pairs

Petra Lueders, Maxim Yulikov, Gunnar Jeschke

Laboratory of Physical Chemistry, ETH Zurich, Switzerland.

Nowadays, site directed spin labelling (SDSL) in combination with pulsed ESR techniques is established to measure distance constraints in the nanometre range and is applied in structure and dynamics studies of biomacromolecules and biomacromolecular complexes. The most common approach is the usage of double electron electron resonance (DEER) on a pair of nitroxide spin labels. In this work we explore an alternative approach to determine distances in the nanometre range based on an orthogonal spin pair consisting of a nitroxide radical and a chelate complex of a gadolinium ion (Gd^{3+}).

DEER measurements on Gd^{3+} - nitroxide spin pairs are best performed at low temperatures (5-10 K) with detection on the central peak of the Gd^{3+} EPR spectrum. The typical offset between pump and observer frequencies is 80 MHz at X band and 290 MHz at Q band. The advantage for the sensitivity due to the higher Boltzmann factor at low temperatures is further aided by the possibility to perform measurements on Gd^{3+} with significantly shorter repetition times. A drawback for the sensitivity is the smaller fraction of Gd^{3+} centres participating in the formation of the DEER echo.

In this arrangement the DEER experiment reveals no orientation selection even if soft pulses are used. The distances and distance distributions obtained from Gd^{3+} - nitroxide DEER are the same for X and Q band.

We also present a first example, where Gd^{3+} - nitroxide DEER and nitroxide – nitroxide DEER can be measured on the same model system. This allows us to make the first approximate comparison of the performance of these two DEER techniques.

We qualitatively discuss new effects supposedly related to the spin dynamics of the Gd^{3+} centres during the DEER experiment and make a first attempt to analyze the performance of Gd^{3+} - nitroxide DEER experiment with respect to the zero field splitting parameters of Gd^{3+}.

We show that even at X band Gd^{3+} - nitroxide DEER can be performed on biologically relevant model systems with concentrations of labelled macromolecules in the range of 100 – 200 μM and with the nitroxide label within a lipid bilayer.

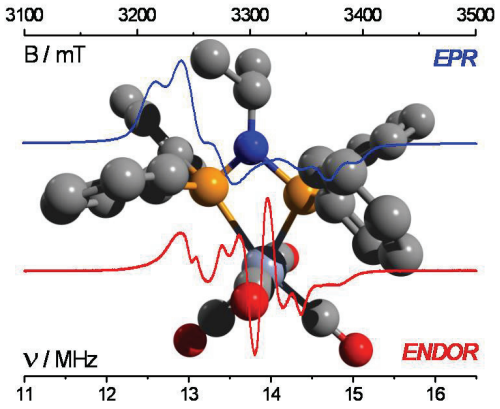
Understanding the role of paramagnetic organometalic redox centres in oligomerisation catalysis by CW and Pulsed EPR & ENDOR spectroscopy

Damien M. Murphy, Emma Carter, Lucia McDyre, Kingsely J. Cavell

School of Chemistry, Cardiff University, Main Building, Park Place, Cardiff CF10 3AT, UK.

Chain growth reactions, such as ethylene oligomerisation, are extremely important in industry since the resulting alpha-olefins can be transformed into a range of commodity chemicals from surfactants to polymer additives. Homogeneous catalysts, based on nickel or chromium organometallic compounds, are widely used for these oligomerisation reactions. Despite the success of these compounds, details of the reaction mechanism are poorly understood, particularly in the case of the Cr based catalysts. A greater understanding of the mechanism, in particular the metal oxidation state, is vital in order to design the next generation of polymerisation catalysts. Currently, a Cr(I)/Cr(III) catalytic cycle is favoured, however, Cr(II)/Cr(IV) and Cr(III)/Cr(V) cycles have also been proposed.

Here we will describe some of our recent key findings using advanced EPR techniques such as CW and Pulsed ENDOR and ESEEM, to characterise a series of novel complexes based on N-heterocyclic carbenes (and phosphines), with low valent Cr(I) centres. The Cr(I)-PNP complexes [1] are presented for comparison with the NHC systems. Several new paramagnetic species have been identified following activation of the Cr(I) complexes with TEA. The nature of the paramagnetic centres formed in the activated catalyst is found to depend on the levels of TEA added. The symmetry, coordination and structure of the Cr(I) pre-catalyst is shown to change dramatically after activation.



[1] L.E. McDyre, T. Hamilton, D.M. Murphy, K.J. Cavell, W.F. Gabrielli, M.J. Hanton, D.M. Smith, *Dalton Trans.,* **2010** (39) 7792-7799.

EPR Studies of Titania Photocatalysts for Organic Synthesis.

S. Dixon and R. F. Howe

Chemistry Department, University of Aberdeen, Meston Walk, Aberdeen.

Synthetic routes in organic chemistry are often environmentally unattractive, sometimes requiring high energy and producing many by products, with varied selectivities and yields. Titania photocatalysts, under UV irradiation, are known to generate free radicals, which may be useful in understanding and developing greener organic synthesis methods, by means of free radical chemistry.

During irradiation, electrons in the TiO_2 are excited from the valence to the conduction band leaving behind positive holes in the valence band, and forming free electrons in the conduction band[1]. These electrons can react with organic substrates, when these are present, driving organic reactions forward.

Using light as a reagent for organic reactions avoids such drastic use of temperature and pressure to force the reaction, and may be a step forward in looking at sustainable organic synthesis with reduced environment impact[2]. Advantageously radical chemistry also allows for increased selectivity within syntheses under mild conditions[3].

Initial results will be reported using acetic acid to produce methyl radicals, which can be clearly assigned from their EPR spectrum, along with their dependence on temperature. This follows the photo-Kolbe reaction pathway involving attack of positive holes onto carboxylic acid molecules[4].

Future work on this project will focus on increasing the complexity of the organic introduced into the reaction, in order to investigate further radical formation and reaction with specific interest in radical addition to alkenes and stereo-controlled alkylations of aldehydes. Understanding the mechanisms involved and the radicals formed will be vital in order to be able to scale up the reactions in the future to provide a more environmental approach to organic synthesis.

This research is supported by the EPSRC, EP/I00372X.

[1] R. F. Howe and M. Gratezel, J. Phys. Chem., 89, 4495, 1985.
[2]A. Albini and M. Fagnoni, " Green Chemical Reactions", Springer, 2008, page 173.
[3] "Radicals in Organic Synthesis" Wiley-VCH, 2001.
[4]B. Kraeutler and A. J. Bard, J. Am. Chem. Soc. 100, 2239, 1978.

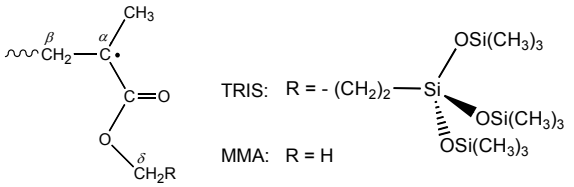
Dynamic effects on the EPR spectra of propagating methacrylic radicals

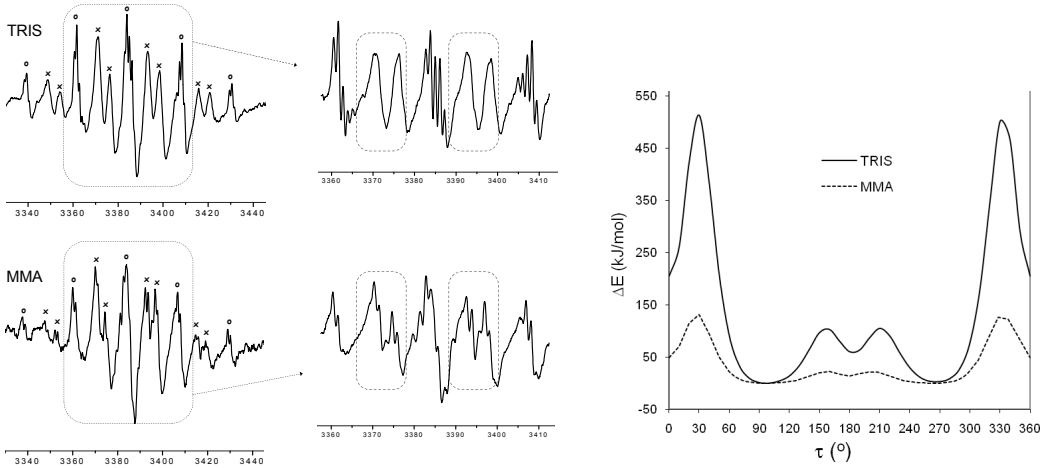
P. Calle, L. Hermosilla, C. Sieiro
Departamento de Química Física Aplicada Facultad de Ciencias
Universidad Autónoma de Madrid, 28049 Madrid, Spain

The EPR spectra of polymethacrylic propagating radicals obtained under high resolution photopolimerization conditions exhibit additional splittings to the 13-line pattern communly observed. The last one is due to the coupling of protons at β position respect to the radical centre, whereas the additional splitting has been assigned to protons located at δ position, in the side chain, as theoretical calculations predict. In most of the experiments, the low concentration of propagating radical during the polymerization process compels to use such a registration conditions that prevent the observation of this coupling.

The extra splitting observed in the EPR spectrum during the photopolimerization of a set of methacrylic monomers shows important features that are function of the nature of the side chain, R, specially the flexibility of the side chain. So, 3-[tris(trimethylsilyloxy) silyl]propyl methacrylate (TRIS), with a big and non-flexible side chain, exhibits an alternating line broadening pattern, under high resolution conditions, whereas the methyl methacrylate (MMA) spectrum, at the same conditions, exhibits δ-splitting in all the lines.

The alternating line width pattern could appear when hyperfine coupling constants are being modulated by conformational motion near the radical centre. Dynamic effects as result of the rotation barriers around of the C_α-C_β bond in methacrylic propagating radicals are being investigated. The structure of the side chain results in an important influence on the torsional barriers for conformational transitions.





EPR study of Mn centres in $PbTiO_3$ single crystals

I. Ahmad[*], S.C. Hogg[*], V.V. Eremkin[**], V. Smotrakov[**], and D.J. Keeble[*]

[*]Carnegie Laboratory of Physics, School of Engineering, Physics, and Mathematics, University of Dundee, Dundee DD1 4HN, UK.
[**]Institute of Physics, Rostov State University, Rostov on Don 344090, Russia

Transition metal ion doping of ferroelectric perovskite oxide, ABO_3, ceramics remains a primary method for engineering modified piezoelectric properties. So-called 'hard' piezoelectric ceramics typically incorporate acceptor ion substitutional dopants, ions with a valence less than the host ion. In the perovskite titanate oxide materials transition metal ions can be substituted for Ti^{4+} at the octahedrally coordinated B-site. Charge compensation must occur, and results in oxygen vacancy formation. This counters the excess negative charge. Further, electrostatic attraction favours the nearest neighbour sites and defect complexes form which exhibit a local electric dipole moment and which can act as pinning sites for domain walls. Iron doping has been extensively studied and EPR has identified the uncompensated Fe^{3+} defect and observed two types of Fe^{3+}–V_O dipole defects [1]. Here we report on EPR studies of Mn doped single crystal $PbTiO_3$.

The as-received $PbTiO_3$:Mn crystals showed spectra from the Mn^{4+} centre only, a complete set of zero field splitting transitions were observed and confirmed the previously reported spin Hamiltonian values [2]. Charge compensation is not required, consistent with the observation of only one Mn EPR centre. Vacuum annealing experiments were performed at temperatures between 450 °C and 600 °C, the ferroelectric transition tetragonal to the cubic, paraelectric, phase occurs at 490 °C. A second Mn centre identified as Mn^{2+} and exhibiting a large zero field splitting was observed and is characterised. The results are compared to those obtained from Fe doped $PbTiO_3$.

I.A. and D.J.K. acknowledge support from EPSRC EP/F039034/1.

[1] D. J. Keeble, M. Loyo-Menoyo, Z. I. Y. Booq, R. R. Garipov, V. V. Eremkin, and V. Smotrakov, *Phys. Rev. B* **80**, 014101 (2009).
[2] D. J. Keeble, Z. Li, and E. H. Poindexter, *J. Phys.: Condens. Matter* **7**, 6327 (1995).

EPR and spin relaxation of charge carriers in π conjugated polymer fullerene blend thin films

D.J. Keeble

Carnegie Laboratory of Physics, School of Engineering, Physics, and Mathematics, University of Dundee, Dundee DD1 4HN, UK.

Recently coherent Rabi oscillations persisting for several microseconds at room temperature have been observed in a π-conjugated polymer – fullerene blend devices using pulsed electrically detected magnetic resonance (EDMR) [1]. Photo-EPR studies on similar blends have identified both the positive hole on the conjugated polymer backbone [2,3], the positive polaron, and the fullerene radial anion [2]. The EDMR spectrum occurs at the *g*-value of the positive polaron, the lack of a contribution at the *g*-value of the fullerene anion supports the conclusion that the EDMR signal is due to spin dependent bipolaron formation associated with polaron transport in the conjugated polymer phase. This work has stimulated interest in the spin relaxation properties of paramagnetic centres in these blends.

Here 9.5 and 35 GHz pulsed EPR measurements on poly(2-methoxy-5-(2'-ethyl)-hexyloxy-*p*-phenylene) vinylene (MEH-PPV) [6,6]-phenyl C_{61}-butyric acid methyl ester (PCBM) dip-cast thin films between 10 – 150 K are reported. The films were illuminated using a 407 nm GaN laser. The *g*-values for the polaron and fullerene centres were confirmed using a C_{60}:N reference sample. Inversion-recovery measurements using a pulse train were used to determine T_1 times for both centres. Primary echo decay was used to measure T_2 relaxation times, the Carr-Purcell-Meiboom-Gill sequence was also used.

D.J.K. acknowledges support from EPSRC EP/F039034/1.

[1] J. Behrends, A. Schnegg, K. Lips, E. A. Thomsen, A. K. Pandey, I. D. W. Samuel, and D. J. Keeble, *Phys. Rev. Lett.* **105**, 176601 (2010).
[2] J. De Ceuster, E. Goovaerts, A. Bouwen, J. C. Hummelen, and V. Dyakonov, *Phys. Rev. B* **64**, 195206 (2001).
[3] A. Aguirre, P. Gast, S. Orlinskii, I. Akimoto, E. J. J. Groenen, H. El Mkami, E. Goovaerts, and S. Van Doorslaer, *Phys.Chem.Chem.Phys*. **10**, 7129 (2008).

Spectroscopic and electrochemical properties of hydroxylated derivatives of 2,6-dimethoxy-1,4-benzoquinone

Haleh H. Haeri[*], Rubin Gulaboski[**], Ivan Bogeski[*], Valentin Mirčeski[***], Markus Hoth[*], Reinhard Kappl[*]

[*] Department of Biophysics, Saarland University, Homburg, Germany
[**] University "Goce Delčev", Stip, Republic of Macedonia
[***] Institute of Chemistry, Faculty of Natural Sciences and Mathematics, "Sv Kiril i Metodij" University, Skopje, Republic of Macedonia

Methoxy-substituted 1,4-benzoquinones are interesting models for the biologically important compounds of the coenzyme Q family, which have a key function in electron-transfer and redox processes. Partially substituted 1,4-benzoquinones are known to be hydroxylated at quinoid positions in alkaline media, forming intermediate semiquinone radicals accessible to EPR spectroscopy.

Recently, we observed that hydroxylation also occurs at the methoxy-substituted positions of partially (2,6-dimethoxy-1,4-benzoquinone, Q0) and, particularly, of fully substituted 1,4-benzoquinones (Q1, Q10) yielding mono- and di-hydroxylated products. EPR and cyclic voltammetry are utilised to investigate the transformation mechanism, the kinetics of radical formation and the electrochemical properties of intermediates and products, focusing here on 2,6-dimethoxy-1,4-benzoquinone (2,6-bq). The experimental results are complemented with DFT calculations.

The transformation of 2,6-bq at pH>12 proceeds via a mono-hydroxylated intermediate inducing a radical on the parental compound. Its spectral EPR signature in liquid state at room temperature is consistent with two OCH_3-groups and two equivalent ring protons. DFT methods indicate that hydrogen bonding is essential to obtain the correct isotropic hyperfine couplings. In the time course of the reaction a secondary radical slowly appears which lacks a methoxy-group, and, on the basis of its couplings, is assigned to the di-hydroxylated form of the compound. From several possible structures, DFT calculations clearly favour one configuration. The loss of an OCH_3-group should be accompanied by formation of methanol, which was demonstrated by NMR. The spectroscopic changes are also monitored in cyclic voltammetry at high pH values. After neutralizing the solvent, the hydroxylated compound reveals a more negative redox potential, and, importantly, has a strong binding capacity for divalent ions such as Ca^{2+} and Ba^{2+} in its reduced state. The 2:1 stoichiometry (Ca^{2+}:2,6-bq) matches with a di-hydroxylated compound and is confirmed by DFT calculations.
The detailed information derived from the model transformation reaction of 2,6-bq will be useful to understand possible reactions of the more complex, biologically relevant quinones.

In Situ ESR Studies of Thermal and UV Degradation of Space-Grade Polymers

Kenneth Rasmussen[*], Günter Grampp[*], Marc van Eesbeek[**], Thomas Rohr[*]

[*]Institute of Physical and Theoretical Chemistry, Graz University of Technology
[**]Materials Space Evaluation & Radiation Effects Section, ESA-ESTEC

Recently, the European Space Agency (ESA) has expanded their testing laboratories by several facilities for *in situ* investigation of samples submitted to simulated space environments. One of these incorporates an ESR spectrometer[1].

In the present contribution, the radicals formed during thermal and VUV degradation of several common space-grade polyimide and silicone materials were studied using ESR spectroscopy. The stability of the materials was assessed and by recording subsequent spectra at a sufficient rate, the degradation kinetics could be followed.

The silicones used included optical adhesives such as DC 93-500 and Elastosil S 690, all which have no inherent ESR signal. Thermal degradation at up to 350°C showed only a slight concentration of radicals for all silicones. UV degradation on the other hand led to different responses of the materials. All exhibited a significant rise in ESR signal over a relatively short period of intense radiation and in some cases hyperfine interactions could be observed.

Several polymer films, including Kapton HN and Upilex S, were also studied [2] and the obtained ESR spectra were in good accordance with previous reports [3,4]. Most of these films exhibited a very good thermal stability which is in accord with findings from other methods [5]. Kapton, however, showed a complex behaviour with at least two separate mechanisms taking place during the thermal treatment. VUV exposure showed an even more rapid degradation of the materials.

Mechanistic details of the *in-situ* response of silicone adhesives and polyimide films to thermal and VUV exposure will be presented and the suitability of ESR spectroscopy for fast screening of materials will be discussed.

[1] K. Rasmussen, G. Grampp, M. v. Eesbeek, T. Rohr, Proceedings of the 11th International Symposium on Materials in a Space Environment, 2009 (Published online at http://esmat.esa.int)
[2] K. Rasmussen, G. Grampp, M. v. Eesbeek, T. Rohr, ACS Appl. Mater. Interfaces, 2 (2010) 1879-1883
[3] M. A. George, B. L. Ramakrishna, W. S. Glausinger, J. Phys. Chem., 94 (1990) 5159-5164
[4] M. K. Ahn, S. S. Eaton, G. R. Eaton, M. A. Meador, Macromolecules, 30 (1997) 8318-8321
[5] C. O. A. Semprimoschnig, S. Heltzel, A. Polsak, M. v. Eesbeek, Proceedings of the 9th ISMSE, 2003, ESA SP-540, 161-167

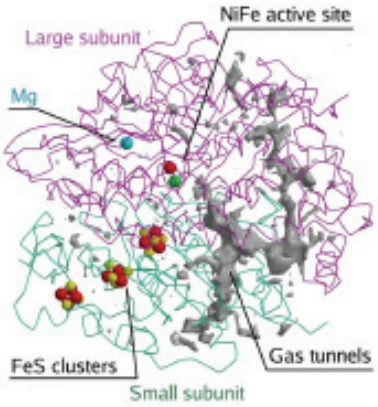
Understanding inhibitor resistance mechanisms of hydrogenases: New insights from site-directed mutagenesis, EPR, and electrochemistry

B. Burlat*, P.P. Liebgott, E. Etienne*, M. Brugna, M. Rousset*, C. Léger*, S. Dementin* & B. Guigliarelli*

*Bioénergétique et Ingénierie des Protéines, BIP-UPR9036, IMM-IFR88, CNRS and Aix-Marseille University, 13402 Marseille, FRANCE

Many microorganisms can use the reversible oxidation of H_2 in their bioenergetic metabolism thanks to hydrogenase enzymes. Owing to their potential applications, these efficient biological catalysts are the subject of extensive studies. However, most hydrogenases are inactivated by dioxygen which critically restricts their use. Thus, understanding and improving O_2 resistance is a prerequisite for their biotechnological applications [1].

A subclass of these enzymes contains a Ni-Fe dinuclear centre as active site that is strongly buried in the protein and connected to the solvent through molecular tunnels. Both substrate (H_2) and inhibitors (O_2, CO) are considered to diffuse through these tunnels to reach the active site. Thus, rational strategies to improve the enzyme resistance were based on site-directed mutagenesis modifications of the tunnels to control the diffusion of inhibitors [2, 3].



In this work, we show that the introduction of sulphur containing amino acid at the end of the tunnel located near the Ni-Fe active site enables a significant increase of inhibitor tolerance [4]. By using a combination of kinetic studies by electrochemistry and EPR spectroscopy, we demonstrate that the improved O_2 resistance is not explained by restrictions of the tunnel size, and that alterations of electron and proton transfers must be taken into account. These findings open new ways for enzyme engineering strategies to improve the hydrogenase properties.

[1] Leroux F, Dementin S, Burlat B. *et al*. *Proc. Natl. Acad. Sci. U S A,* 105, 11188-93, **2008**
[2] Dementin S., F. Leroux, L. Cournac, *et al*., *J. Am. Chem. Soc*. 131, 10156-10164, **2009**
[3] Liebgott P.-P., Leroux F, Burlat B. *et al., Nat. Chem. Biol* 6(1) 63-70, **2010**
[4] Liebgott, P.P, de Lacey, A.L., Burlat, B. *et al., J. Am. Chem. Soc*., **133,** 986-997**, 2011**

ENDOR measurements on *Arabidopsis thaliana* Cryptochrome-1

Ringo Wenzel[*] Margaret Ahmad[**] and Robert Bittl[*+]

[*]Free University of Berlin, FB Physik, Berlin
[**]University of Paris IV, FRE-CNRS-2846
[+]Correspondence to robert.bittl@fu-berlin.de

Cryptochromes are blue-light sensing photoreceptors found in plants, animals and humans, that share a high degree of structural and sequence homology with photolyases, and contain the same flavine cofactor.[1] Yet, cryptochromes usually do not show a DNA-repair function and their mechanism of action is basically unknown. The function is generally accepted to be a trigger to the circadian rhythm[2] while additional ideas (magnetic sensor in migratory birds, initiation of flowering in plants) are still under discussion.

It could be shown, that blue light irradiation leads to an accumulation of the semi-reduced flavin radical state, while the fully oxidized flavin was found to be the dark stable ground state.[3] Nevertheless, the activation process, as well as a possible role of the fully reduced state, is still not known.

For measuring the cryptochrome mechanism, electron paramagnetic resonance (EPR) provides a powerful tool. Since EPR-experiments are sensitive to radicals only, the semi-reduced flavin state can be probed exclusively. Additionally, it can be applied not only to purified protein, but also to whole cells, as shown in [4].

Here, we applied orientation selective ENDOR-EPR measurements to *Arabidopsis* Cry1 in overexpressing Sf21 insect cells and purified protein to characterize the flavin redox state and elucidate differences between those two systems. As a result, we report a significant shift of the larger H(1')-coupling, which indicates a small geometric reorientation of the flavin cofactor during the process of purification.

References

[1] Malhotra, K., Kim, S. T., Batschauer, A, Dawut, L, Sancar, A. (1995) *Biochemistry* **34**, 6892

[2] A. Sancar (2004) *Journal of Biological Chemistry* **279**, 34079

[3] Banerjee, R., Schleicher, E., Meier, S., Viana, R. M., pokorny, R., Ahmad, M., Bittl, R., Batschauer, A. (2007) *Journal of Biological Chemistry* **282**, 14916

[4] Bouly, J.-P., Schleicher, E., Dionisio-Sese, M., Vandenbussche, F., Van Der Straeten, D., Bakrim, N., Meier, S., Batschauer, A., Galland, P., Bittl, R., Ahmad, M. (2007) *Journal of Biological Chemistry* **282**, 9383

Assessing Conformational States of MscS with X- and W-band PELDOR

R. Ward*, C. Pliotas*, E. Brennigan*, G. Hagelueken*, R. Hunter**, H. ElMkami**, G. M. Smith**, J. H. Naismith*, O. Schiemann*

*Biomedical Sciences Research Complex, University of St Andrews, St Andrews, UK.
**School of Physics and Astronomy, University of St Andrews, St Andrews, UK.

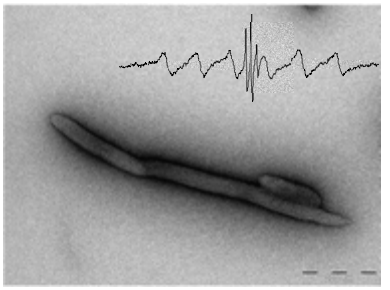
We seek to unravel the conformational states of the mechanosensitive channel of small conductance (MscS) during opening and closing. To achieve this, we join site-directed spin labelling with Pulsed Electron-Electron Double Resonance (PELDOR) and other biophysical methods.

MscS is a homo heptamer meaning that labelling each monomer with a single label yields seven in the heptamer. In initial experiments, we aimed at obtaining X-band PELDOR time traces with resolved modulation. We approached this by finding the right sample conditions for MscS solubilised in detergent. Adjusting the expression, purification and labelling conditions as well as the sample buffer conditions finally lead to reproducible sample conditions yielding resolved modulations in the time traces. The importance to check these samples for functionality of the channel using electro-chemical patch-clamp techniques will be highlighted. In the next step we wanted to get in addition to distance information also orientation information and measured the same samples at W-band frequencies using the HIPER machine developed in the lab of G. M. Smith. Taken together this approach enables a reliable mapping of conformational states.

This research is supported by the BBSRC and the EPSRC.

DNA Condensation observed by Multi-Spin-Probe Techniques

Dennis Kurzbach[*], Caroline Velte[*], Philipp Arnold[#], Dariush Hinderberger [*]
[*]Max Planck Institute for Polymer Research, Ackermannweg 10, 55128 Mainz.
[#]Johannes Gutenberg-University Mainz, Institute for Zoology, Johannes-von-Müller Weg 6 , 55128 Mainz.
Corresponding contact: kurzbach@mpip-mainz.mpg.de



TEM picture of rod-like DNA condensates induced by a Newkome-type dendrimer with spermine end-groups. (Charge ratio (c_+/c_-) = 2.3) The ESR signal composed of the labelled denrimer and the Mn^{2+} signal is shown above.

Here we present CW (continuous wave) electron paramagnetic resonance (EPR) and Transmission electron microscopy (TEM) data that characterize DNA condensation [1] by multi-spin-probe techniques. Spin labelled, positively charged condensation agents (spermine-terminated Newkome-type G2 dendrimers with 27 positive charges per molecule) are able to neutralize > 90% of the negative charge of a DNA (884bp) double strand and thereby condense DNA into tightly packed structures. At a ratio of 2.3 of positive charges of the dendrimers to negative charges of the DNA strands the condensates (as seen by TEM) have rod-like shape while other charge ratios lead to less well defined aggregates (observed by TEM). Adding small concentrations of monovalent salt resulted in aggregation of the rod-like condensates and partial transformation to toroids. Mn(II) ions added to the buffered DNA solution only gave rise to a Mn^{2+} EPR signal, when the charge ratio was close to 2.3. Therefore the structure of the DNA condensates/aggregates in the solid state could be monitored in solution by the Mn(II) signal. Hence, the observation of the Mn^{2+} signal is indicative for the rod-like shape in the condensate. At other charge ratios then 2.3 the Mn(II) ions are bound to the DNA strands and as a consequence their EPR signals become too broad to be detected.
Comparison with DNA condensation and polyelectrolyte theory [2] indicates that a charge inversion of the DNA strands could explain the behaviour of the Mn(II) signal. In accordance with theoretical DNA concepts, the manganese signal becomes stronger with increasing temperature and disappears at monovalent salt concentrations above 35 mM.
The generation-two, spermine terminated condensation agent was monitored in CW EPR through its spin label. CW EPR proves that the condensation agent sticks tightly to the DNA strands regardless of ionic strength concentration or temperature. Therefore all observed answers of the system to stimuli like temperature or salt concentration can be ascribed to interactions between dendriplexes of DNA and dendrons and not to interactions between the condensation agents and individual double-stranded DNA.

1. Bloomfield, V.A., Biopolymers, 1997. **44**: p. 269-282.
2. Grosberg, A.Y., T.T. Nguyen, and B.I. Shklovskii, Review of Modern Physics, 2002. **74**: p. 329-345.

Combining NMR docking data with EPR distances and *in silico* calculations for a more complete model of colicin protein-protein interactions

Morgan Bye*, Alicia-Anne Stratford*, Geoff Moore, Fraser MacMillan*

* Henry Wellcome Unit for Biological EPR, University of East Anglia, Norwich, UK
School of Chemistry, University of East Anglia, Norwich, UK

Currently atomic resolution structures of protein-protein interactions are commonly limited to X-ray crystallography with its unnatural buffers, pHs and forced crystalline structure, while NMR developments have allowed for solution based structures of small complexes to be developed [1] however *ab initio* information still remains a developing field [2,3].

Site-directed spin labelling (SDSL) in combination with electron paramagnetic resonance (EPR) allows for the precise measurement of intra and intermolecular distances. Such distances traditionally are obtained using pulsed electron double resonance (PELDOR) techniques. New *in silico* methods now also allow for the comparison of computation and experimental data [4].

Using SDSL and distance measurements at several sites within a protein-protein complex allows for a three dimensional model to be built up using distance constraints, which when combined with NMR docking data and software gives a comprehensive model [5].

Using the well-characterised system, of colicin E9 and its cognate inhibitor, Im9 [6] a comprehensive solution is being developed to combine such NMR docking models, with PELDOR distance (and angle) constraints and *in silico* computations which are comparable to X-ray crystallographic models.

With this technique in place the model will be expanded to characterise other biologically relevant protein-protein interactions including the ill-defined non-cognate binding pairs of colicin E9 with the inhibitor proteins, Im2, Im7 and Im8.

[1] Ferentz et al, Rev Biophys, 33, 29 (2000)
[2] Palma et al, Proteins, 39, 372 (2000)
[3] de Vries et al, Proteins, 69, 726 (2007)
[4] MMM - http://www.epr.ethz.ch/software/index
[5] HADDOCK - http://www.nmr.chem.uu.nl/haddock/
[6] Cascales et al, MMBR, 71, 1 (2007)

Electron Spin Resonance Studies on Copper Triangles.

Eike T. Spielberg[*], Christopher J. Wedge[*], Daniel Plaul[**], Winfried Plass[**], Arzhang Ardavan[*]

[*] CAESR, Department of Physics, Clarendon Laboratory, Parks Road, Oxford, OX1 3PU, United Kingdom.
[**] Institute for Inorganic and Analytical Chemistry, Friedrich-Schiller-University, Carl-Zeiss-Promenade 10, Jena, Germany.

We performed a series of ESR investigations on trinuclear copper complexes. These compounds show strong antiferromagnetic interactions between the copper ions, which give rise to a spin frustrated system comprising a degenerate S = ½ ground state [1]. The degeneracy can be lifted by symmetry lowering and/or antisymmetric exchange interactions commonly referred to as Dzyalosinsky-Moriya (DM) interactions [2]. In comparison with other suitable transition metal complexes proposed as quantum bits [3], these systems feature additional promising properties. For instance, the two chiral ground states are mixed by an externally-applied electric field allowing for fast manipulation with high spatial resolution [4].

To obtain a good thermal isolation of the active ground states, very strong exchange interactions are required. Complexes based on triaminoguanidines show a reliable formation of trinuclear complexes and exhibit very strong antiferromagnetic interactions as well [5]. These systems have been investigated due to their electronic ground state and their relaxation properties. We show that only weak lifting of degeneracy occurs by means of symmetry lowering and DM interactions. They combine long T_1 times with intermediate T_2 times.

This research is supported by EPSRC and the EU. E.T.S. acknowledges financial support by a DFG Research Fellowship. A.A. is supported by the Royal Society.

[1] J. Yoon et al., *J. Am. Chem. Soc.* **2004**, *126*, 12586.
[2] a) I. J. Dzyalosinski, *J. Phys. Chem. Sol.* **1984**, *4*, 241; b) T. Moriya, *Phys. Rev. Lett.* **1960**, *4*, 228; c) T. Moriya, *Phys. Rev. 1960*, *120*, 91.
[3] a) A. Ardavan et al. , *Phys. Rev. Lett.* **2007**, *98*, 057201; b) R. E. P. Winpenny, *Angew. Chem. Int. Ed.* **2008**, *47*, 7992.
[4] a) M. Trif, et al., Phys. Rev. Lett. **2008**, *101*, 217201; b) M. Trif et al., Phys. Rev. B **2010**, *82*, 045429.
[5] a) A. E. Ion et al., *Inorg. Chim. Acta* **2007**, *360*, 3925; b) A. Zharkouskaya et al., *Chem. Comm.* **2005**, *8*, 1145; c) A. Zharkouskaya et al., *Eur. J. Inorg. Chem.* **2005**, *24*, 4875.

An EPR/ENDOR study of free radicals in X-ray and plasma irradiated polymer powders.

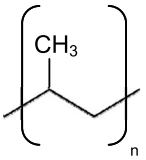
I. Caretti[*], S. Van Doorslaer[**] and I. Jiménez[*]

[*] Instituto de Ciencia de Materiales de Madrid (ICMM-CSIC), Campus de Cantoblanco, 28049 Madrid (Spain)
[**] University of Antwerp, Department of Physics, Universiteitsplein 1 (Building N), 2610 Wilrijk, Belgium

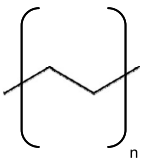
Free radicals, both as intermediate and stable paramagnetic species, are readily formed in polymers by means of ionizing radiation. The chemical changes induced in this way to the polymer result often in the modification of its properties, mainly through cross-linking, degradation and grafting mechanisms. Even though polymer irradiation with gamma rays, X-rays, UV light or high energy particles is regularly applied for many technological applications, identification of the radicals formed is still the subject of active debate.

In this work, we investigate x-ray irradiated polypropylene (PP), polyethylene (PE), polyamide nylon-6 (N6) and polyimide (kapton®) polymer powders prepared by cryogenic grinding at liquid N_2 temperature. In case of PP, a solution-precipitation procedure was also carried out to produce a powder with smaller particle size. The latter was surface-modified for 30s using N_2, O_2 and Ar plasmas created inside a low pressure radiofrequency plasma reactor.

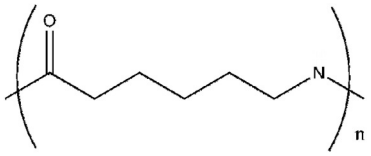
Mainly continuous wave (CW) EPR/ENDOR methods have been reported for the study of organic radicals in irradiated polymers, while the corresponding pulsed techniques have been scarcely used. Here, we have exploited the combination of CW and pulsed X-band EPR, CW W-band EPR and X-band Mims ENDOR to explore the formation of alkyl and peroxide radicals by irradiation and probe the weak interactions with neighbouring protons, as well as nitrogens in N6 and kapton. Our results are examined in the context of currently available structural models for this type of radicals.



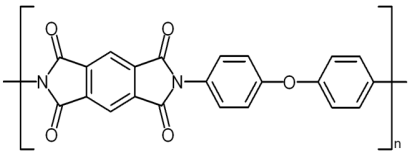
PP(Polypropylene)



PE(Polyethylene)



NYLON 6 (polycaprolactam)



KAPTON (poly(4,4'-oxydiphenylene-pyromellitimide)

Coordination isomers of histidylglycine copper(II)complex investigated by temperature dependent CW ESR and CD spectra.

Nóra Veronika Nagy[*], Béla Gyurcsik[**], Eszter Tóth[**] and Antal Rockenbauer[*]

[*]Institute of Structural Chemistry, Chemical Research Center of the Hungarian Academy of Sciences, Budapest, Hungary
[**]Department of Inorganic and Analytical Chemistry, University of Szeged, Hungary

The side-chain and backbone donor atoms of peptides are frequently in competition for the binding sites of metal ions, and so complexes with same composition but different structures (isomers) can be formed. In aqueous solution of histidylglycine and copper(II) the coexistence of two alternative (peptide- and histidine-type) coordination have already been detected in the complex of $[CuL]^+$ (Figure 1) [1]. In this work our goal was to give further evidence for the existence of this isomer equilibrium and determine the thermodynamic parameters by the help of temperature dependent CW ESR and CD methods. (As higher CD effect was expected from histidylalanine than from histidylglycine, the former was applied for CD measurements.)

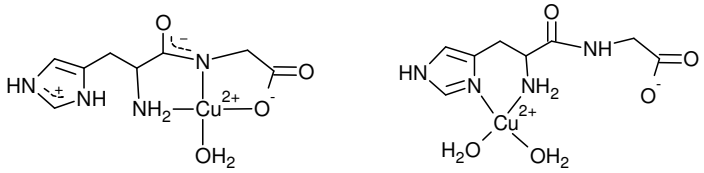


Figure 1. Peptide-type (left) and histidine-type (right) coordination in the $[CuL]^+$ complex of histidylglycine (L)

In both cases ten spectra were recorded between 283 – 328 K in steps of 5 K. The series of EPR spectra were then evaluated by a "two-dimensional" simulation program [2] which involves the simultaneous analysis of all ESR spectra recorded at various temperatures. The program adjusts the isotropic ESR parameters of the species taking into account their temperature dependence and determines also the thermodynamic parameters. The alteration of the isomer ratio with temperature have been taken into account by the Van't Hoff relation ($\ln K = -\Delta H / RT + \Delta S / R$). Decomposition of temperature dependent CD spectra is in accordance with the ESR results.

References

[1] T. Szabó-Plánka, N. V. Nagy, A. Rockenbauer and L. Korecz, *Inorg. Chem*. 41, 3483-3490 **2002**
[2] A. Rockenbauer, N. V. Nagy, F. Le Moigne, D. Gigmes and P. Tordo, *J. Phys. Chem. A* 110, 9542-9548, **2006**

Acknowledgments
We thank the Hungarian Scientific Research Found (OTKA, Grant F67581 and K72781) and GVOP-3.2.1.-2004-04-0214/3.0 for financial support.

Conformational Changes Underlying Calcium/Calmodulin-Dependent Protein Kinase II Activation

Laurel Hoffman[*], Roger Colbran[*], and Hassane Mchaourab[*]

[*] Department of Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, Nashville, Tennessee, 37232, USA

We used spin labeling and electron paramagnetic resonance (EPR) spectroscopy to elucidate the structural and dynamic bases of autoinhibition and activation of calcium/calmodulin-dependent protein kinase II (CaMKII). CaMKII interprets information conveyed by the amplitude and frequency of calcium transients by a controlled transition from an autoinhibited basal intermediate to an autonomously active phosphorylated intermediate. While calmodulin (CaM) binding, autophosphorylation, and catalytic-regulatory domain interactions referred to as autoinhibition have been linked to CaMKII function, the underlying structural and dynamic framework of activation is poorly understood. Here we utilize the continuous wave EPR parameter of mobility and distances extrapolated from pulsed EPR experiments to describe the enzyme's conformation in various catalytic intermediates. We find that autoinhibition involves a conformeric equilibrium of the regulatory domain, modulating substrate and nucleotide access. Binding of calmodulin to the regulatory domain induces conformational changes that release the catalytic cleft, activating the kinase and exposing an otherwise inaccessible phosphorylation site, threonine 286. Autophosphorylation at Thr286 further disrupts the interactions between the catalytic and regulatory domains, enhancing the interaction with calmodulin, but maintains the regulatory domain in a dynamic unstructured conformation following dissociation of calmodulin, sustaining activation.

Pulsed Electron-Electron Double Resonance (PELDOR) Distance Measurements in Detergent Micelles

Bela E. Bode[*,**], Reza Dastvan[*], Thomas F. Prisner[*]

[*]Institute of Physical and Theoretical Chemistry and Center for Biomolecular Magnetic Resonance, Goethe University, Max-von-Laue-Strasse 7, 60438 Frankfurt am Main, Germany.
[**]Leiden Institute of Chemistry, Leiden University , Einsteinweg 55, 2333 CC Leiden, The Netherlands.

Pulsed electron−electron double resonance (PELDOR) spectroscopy [1] is a powerful tool for measuring nanometer distances in spin-labeled systems [2,3]. A common approach is doubly covalent spin-labeling of a macromolecule and measurement of the inter-spin distance, or to use singly labeled components of a system that forms aggregates or oligomers [4]. This situation has been described as a spin-cluster. The PELDOR signal, however, does not only contain the desired dipolar coupling between the spin-labels of the molecule or cluster under study. In samples of finite concentration the dipolar coupling between the spin-labels of the randomly distributed molecules or spin-clusters also contributes significantly. In homogeneous frozen solutions or lipid vesicle membranes [5] this second contribution can be considered to be an exponential or stretched exponential decay, respectively.
In this study, we show that this assumption is not valid in detergent micelles [6,7]. Spin-labeled fatty acids that are randomly partitioned into different detergent micelles give rise to PELDOR time traces which clearly deviate from stretched exponential decays. The obtained signals can be modeled quantitatively based on the size of the micelles, their aggregation number, the spin-label concentration and the degree of spin-labeling. As a main conclusion a PELDOR signal deviating from a stretched exponential decay does not necessarily prove the observation of specific distance information on the molecule or cluster. These results are important for the interpretation of PELDOR experiments on membrane proteins or lipophilic peptides solubilized in detergent micelles or small vesicles, which often do not show pronounced dipolar oscillations in their time traces.

[1] A.D. Milov, K.M. Salikhov, M.D. Shirov, *Fiz. Tverd. Tela* **1981**, *23*, 975.
[2] G. Jeschke, Y. Polyhach, *Phys. Chem. Chem. Phys.* **2007**, *9*, 1895.
[3] O. Schiemann, T.F. Prisner, *Quart. Rev. Biophys.* **2007**, *40*, 1.
[4] P. Zou, M. Bortolus, H.S. Mchaourab, *J. Mol. Biol.* **2009**, *393*, 586.
[5] A.D. Milov, D.A. Erilov, E.S. Salnikov, Y.D. Tsvetkov, F. Formaggio, C. Toniolo, J. Raap, *Phys. Chem. Chem. Phys.* **2005**, *7*, 1794.
[6] S. Ruthstein, A. Potapov, A.M. Raitsimring, and D. Goldfarb, *J. Phys. Chem. B* **2005**, *109*, 22843.
[7] B.E. Bode, R. Dastvan, T.F. Prisner, *J. Magn. Reson.* **2011**, doi: 10.1016/j.jmr.2011.03.014.

Using ENDOR and HYSCORE to determine the orientation of the g-tensor in *palustrisredoxin*

Joe Abdalla, Alice Bowen, Janet Lovett, Christiane R Timmel, Stephen Bell, Nicki Hoskins,

Luet Lok Wong and Jeffrey Harmer

Centre for Advanced Electron Spin Resonance, Department of Chemistry, University of Oxford, South Parks Road, Oxford, OX1 3QR

Palustrisredoxin(Pux) is a redox active ferredoxin protein, containing a [2Fe-2S] cluster, which is part of a three protein electron transport chain : ferredoxin reductase, ferredoxin and a cytochrome P450 enzyme. Knowledge of the g-tensor orientation is crucial for our structural DEER studies of the docked complexes formed between proteins in this system. The [2Fe-2S] cluster in the ferredoxin is ligated by four cysteine residues and falls into the 'adrenodoxin' class of iron-sulphur proteins. The orientation of the g-tensor with respect to the molecular frame in such proteins has been discussed in several papers[1], most notably by Kappl *et al*[2] They found that the orientation of $g_{\|}$ is almost perpendicular to the [2Fe-2S] plane, with a slight tilt towards the sulphur of one of the ligating cysteines.

In this work, *Palustrisredoxin* has been analysed using orientationally selective ENDOR spectroscopy at both X- and W- band, together with X-band HYSCORE measurements. We have used a combination of Easyspin and in-house written software to simulate the ENDOR and HYSCORE spectra. The one-electron reduced, paramagnetic [2Fe-2S] cluster (S=1/2) exhibits a large variety of hyperfine couplings to surrounding protons, rendering analysis challenging. In our models, we have used the known crystal structure of *Palustrisredoxin* for structural information on the system, and the results of DFT calculations[3] in order to estimate the distribution of spin density around the [2Fe-2S] centre. We report good agreement with the work of Kappl *et al.*[3], and are able to demonstrate that the use of HYSCORE and ENDOR data provides a powerful combination to allow characterisation of the system's g-tensor orientation and spin density distribution.

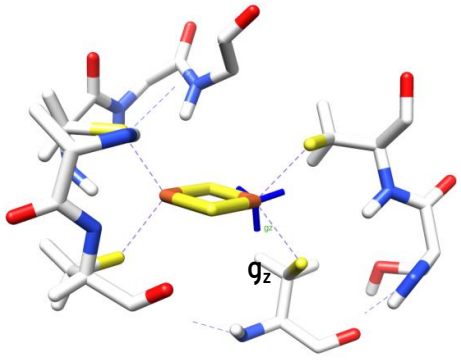


Fig 1. The calculated orientation of the g-tensor in Palustrisredoxin with respect to the molecular frame of the protein

References

1. C. Canne, M. Ebelhauser, E. Gay, J. Shergill, R. Cammack, R. Kappl and J. Hutterman., *Journal of Biological Inorganic Chemistry, 2000, 514-526.*
2. R. Kappl , M. Ebelhauser, F. Hannemann, R. Bernhardt and J. Hutterman, *Applied Magnetic Resonance, 2006, 427-459*
3. L. Noodleman, C.Y. Peng, D.A. Case and J-M. Mouesca, *Coordination Chemistry Reviews, 1995, 199-244*

Spectroscopic properties and electronic structure of the radical ions of cycloheptatrienyl molybdenum and tungsten complexes

Ruth Edge,[*] Emma Fitzgerald,[*] Hannah N. Roberts,[*] Neil J. Brown,[**] Daniel O. Sells,[*] David Collison,[*] Eric J.L. McInnes,[*] Paul J. Low,[**] and Mark W. Whiteley[*]

[*] School of Chemistry, The University of Manchester, Oxford Road, Manchester, M13 9PL, UK
[**] Department of Chemistry, Durham University, South Road, Durham, DH1 3LE, UK

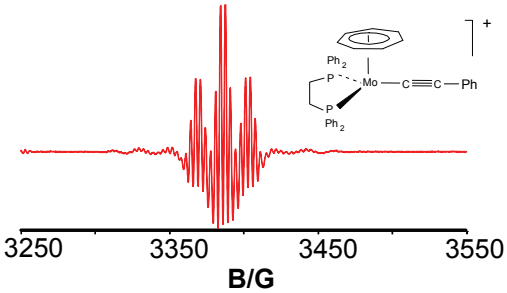
Cycloheptatrienyl (η-C_7H_7) molybdenum and tungsten complexes are isoelectronic with the widely studied iron and ruthenium cyclopentadienyl (Cp/Cp*) complexes and are of interest as 'molecular wires' for their potential use in the development of molecular electronics. They can be easily one-electron oxidised to their, relatively stable, corresponding radical cations, and bimetallic complexes can undergo a reversible, stepwise two-electron oxidation.

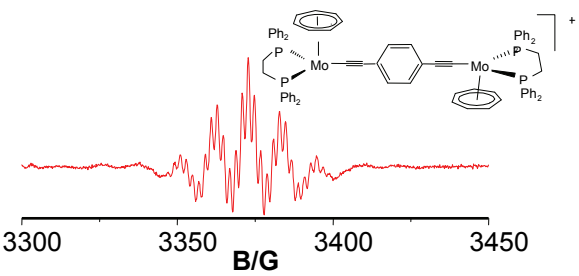
An X-band EPR study of a range of complexes has been undertaken, generating the ions either electrochemically or using a chemical oxidant. Monometallic Mo and W complexes, as well as bimetallic Mo complexes and a Moη-C_7H_7-FeCp* complex have been studied, probing the spin density distribution on the metal and supporting alkynyl derived ligands.

Optimal resolution of the fluid solution EPR spectra was achieved for the Mo complexes at *ca.* 243 K and the W complexes at *ca.* 193 K. Complexes of both metals showed well-resolved spectra for molybdenum or tungsten isotopes split by the ligand hyperfine interactions. The molybdenum and tungsten hyperfine splittings could be easily obtained from the first derivative spectra. However, second derivative spectra were recorded in order better to observe and then to simulate the hyperfine splittings to the ligands. The W complexes showed slightly less well resolved spectra than their Mo analogues, with a shift to lower g_{iso} (consistent with the larger spin-orbit coupling constant of W).

Frozen solution spectra at X-band showed no evidence of g-anisotropy, but very small g-anisotropy can be observed at W-band. This is in contrast to Fe and RuCp* complexes, where large g-anisotropy is observed. The frozen solution spectrum of the radical cation of the Moη-C_7H_7-FeCp* complex shows a superposition of the separate contributions of the Moη-C_7H_7 and FeCp* ends of the complex.

Example 2[nd] derivative X-band spectra of monometallic and bimetallic Mo radical cation complexes in CH_2Cl_2 are given below, showing how the coupling pattern is modified from 2×[31]P to 4×[31]P splitting (triplet to quintet), indicating that there is spin delocalisation over both metal centres in the bimetallic complexes.



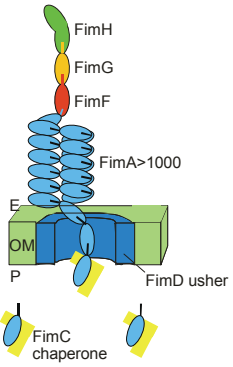


Polymerisation mechanism at the outer membrane usher of Type 1 pili from *E.coli* investigated by SDSL-EPR

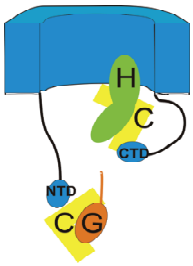
Katharina F. Pirker[*], William J. Allen[**], Micha B. A. Kunze[*], Gabriel Waksman[*,**] and Christopher W. M. Kay[*]

[*] Institute of Structural & Molecular Biology, University College London, Gower Street, London WC1E 6BT, UK.
[**] Institute of Structural & Molecular Biology, Birkbeck College, London WC1E 7HX, UK.

Bacterial infection by uropathogenic *Escherichia coli* (UPEC) is the primary cause of urinary tract infections in Europe and North America and affects many individuals; especially women. There is an increase in resistance to antibiotics by these bacteria and studies of the onset of bacterial infection are gaining importance. Infection of host cells by these bacteria is mediated by fibers (also called *pili*) which are assembled by the chaperone-usher (CU) pathway (Scheme 1). The surface fiber type 1 *pili* are important attachment devices that target UPEC to the bladder epithelium and are encoded by the *fim* gene cluster (*fimA-I*). Type 1 *pili* are thus major virulence factors in the onset of cystitis. These pili are assembled by the chaperone-usher pathway, involving a periplasmic chaperone (FimC) and an outer-membrane assembly platform, the usher (FimD). Chaperone-subunit complexes are recruited by the N-terminal domain (NTD) of the usher and the usher orchestrates the ordered polymerisation (via donor-strand-exchange reaction, DSE) and secretion of subunits.



Scheme 1



Scheme 2

We are using site-directed spin labelling (SDSL) in combination with continuous wave (cw) and pulsed electron paramagnetic resonance (EPR) spectroscopy to investigate the polymerisation mechanism at the usher. Starting with the usher-chaperone-subunit complex FimD:FimC:FimH, the next chaperone-subunit FimC:FimG is added. Spin labels are positioned on the C-terminal domain (CTD) of the usher FimD and the chaperone FimC of FimC:FimG. By measuring distances between spin labels, we could show by EPR that after the DSE reaction, the subunit FimG binds to the CTD. This result is comparable to the recently solved crystal structure of FimD:FimC:FimH where the previous subunit FimH is bound to the CTD of the usher. The involvement of the NTD in the recruitment of the subunits is still not understood [1]. We are now investigating the intermediate state of the FimD:FimC:FimH:FimG complex, right after the addition of the subunit FimG and before it undergoes DSE, i.e. binding to the previous subunit FimH. A plausible intermediate state is suggested in the Scheme 2.

[1] Phan et al., *"Crystal structure of the FimD usher bound to its cognate FimC:FimH substrate"*, (2011) submitted.

Generation and detection of an entangled state for synthetic electron spin-qubits

Shigeaki Nakazawa,[1,8] Robert Hunter[2], Hassan El Mkami[2], Graham Smith[2], Kazunobu Sato,[1,8] Robabeh Rahimi,[1] Tomohiro Yoshino,[1] Shinsuke Nishida,[1,8] Tomoaki Ise,[1,8] Nobuyuki Mori,[1] Yasushi Morita,[3,8] Kazuo Toyota,[1,8] Daisuke Shiomi,[1,8] Kazuhiro Nakasuji,[4] Masahiro Kitagawa,[5,8] Hideyuki Hara,[6] Patrick Carl,[7] Peter Höfer,[7] and Takeji Takui[1,8]

[1]Graduate School of Science, Osaka City University, Osaka 558-8585, JAPAN;
[2]School of physics and Astronomy, University of St. Andrews, St Andrews KY16 9SS, UK
[3]Graduate School of Science, Osaka University, Toyonaka 560-0043, JAPAN;
[4]Fukui University of Technology, Fukui 910-8505, JAPAN;
[5]Graduate School of Engineering Science, Osaka University, Toyonaka, 560-8531, JAPAN;
[6]Bruker Biospin K. K., JAPAN; [7]Bruker Biospin GmbH, GERMANY;[8]CREST-JST, JAPAN

Recently, quantum information science has emerged as a rapidly developing interdisciplinary area since Shor's algorithm was first reported. In particular, the physical realization of scalable quantum computers (QCs) and quantum simulators has been the focus of much current research, in order to accelerate experimental verifications of various theoretical ideas[1,2]. One challenging issue is the implementation of quantum operations and the generation of the entangled state in molecular electron spin qubit systems. One promising approach in QC/QIP experiments is based on novel pulsed electron multiple spin resonance setups such as coherent-dual pulsed ELDOR (ELectron-electron DOuble Resonance) spectroscopy on electron-electron exchange-coupled systems. In this work, for the first time, we have designed and prepared a weakly-coupled stable biradical, as synthetic electron spin-qubits in a magnetically dilute single crystal environment, where the electrons are also hyperfine-coupled at the nitrogen nuclear sites. The molecular design is based on *g*-tensor engineering in oriented systems. The *g* tensors and the *A* tensors of the nitrogen nucleus were determined by the angular dependence of CW-ESR spectra for the diluted single crystal. ESR transitions originating from the non-equivalent qubits in the biradical molecule were well separated, as expected from the crystal symmetry, which means that each qubit is addressable. We have also carried out Q-band pulsed ELDOR measurements for the system to determine the values for intramolecular magnetic dipole-dipole interaction and exchange interaction. Finally, we illustrate that a CNOT quantum gate composed of the electron spin qubits is established by using the synthetic biradical qubits in ensemble. We also show preliminary experiments to generate and detect the entangled state between two electron spin qubits by W-band dual-coherent pulsed ELDOR spectroscopy.

References

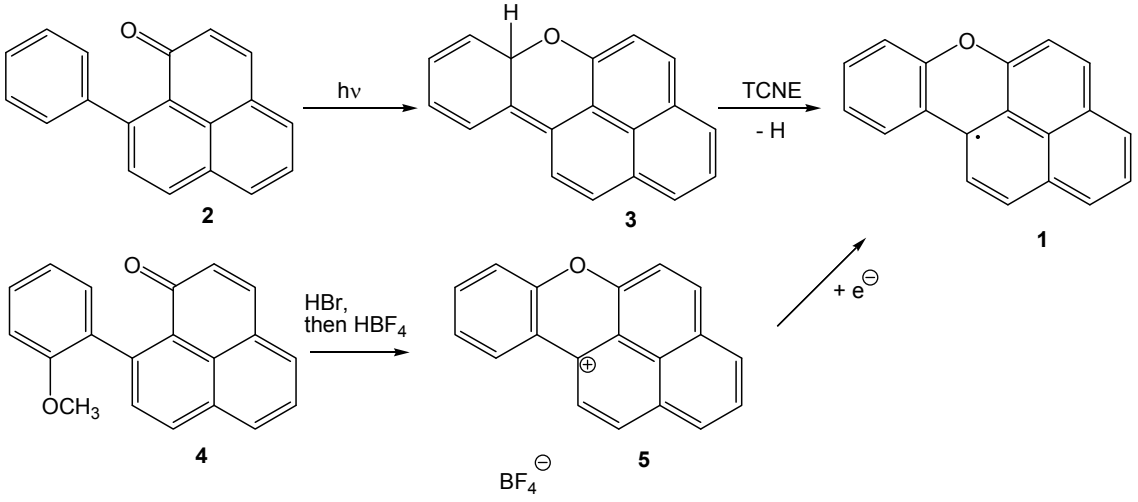
[1] K. Sato, S. Nakazawa, R. Rahimi, T. Ise, S. Nishida, T. Yoshino, N. Mori, K. Toyota, D. Shiomi, Y. Yakiyama, Y. Morita, M. Kitagawa, K. Nakasuji, M. Nakahara, H. Hara, P. Carl, P. Hoefer, and T. Takui, *J. Mater. Chem.*, **2009**, *19*,.3739-3754 (2009).
[2] Y. Morita, Y. Yakiyama, S. Nakazawa, T. Murata, T. Ise, D. Hashizume, D. Shiomi, K. Sato, M. Kitagawa, K. Nakasuji, and T. Takui, *J. Am. Chem. Soc.*, **2010**, *132*, 6944-6946.

Naphthoxanthenyl: An Unusually Stable Carbon-Centered Free Radical

Ommid Anamimoghadam,[1] Cristina Flors,[2] Santi Nonell,[2] and Götz Bucher[1]*

[1]WestCHEM, School of Chemistry, University of Glasgow, Glasgow G12 8QQ, UK.
[2]Grup d'Enginyeria Molecular, Institut Químic de Sarrià, Universitat Ramon Llull, E-08017, Barcelona, Spain.

We report on the synthesis and characterisation of naphthoxanthenyl **1**, a novel carbon-centered radical. Radical **1** can be generated using two different approaches. Photocyclisation of 9-phenylphenalenone **2** results in formation of a short-lived (τ = 10 μs) reaction product **3**. Hydrogen abstraction from **3** by TCNE yields radical **1**, which can be characterised by UV/Vis and ESR spectroscopy. An independent synthesis of 1 is achieved starting from 9-(2-methoxyphenyl)phenalenone **4**, which upon treatment with HBr and counterion exchange with HBF_4 affords naphthoxanthenium tetrafluoroborate **5**. Reduction of **5** yields radical **1** as dark-green solid with bronze lustre. In the solid state, **1** shows a very broad ESR signal devoid of any hyperfine coupling. A highly resolved ESR spectrum can be recorded in dilute benzene solution. Radical **1** is persistent in solution at ambient temperature, and in the solid state and molten up to T = 230 °C, even under air.



The measurement of distances between paramagnetic centers in metalloproteins

M. Krzyaniak[*], P. R. Vennam[*], A. G. Maryasov[**], M. K. Bowman[*]

[*]Department of Chemistry, The University of Alabama.
[**]Institute of Chemical Kinetics and Combustion, Russian Academy of Science.

Dynamic spin-spin interactions are increasingly used in metalloproteins to measure distances between co-factors. The spin-lattice relaxation of one paramagnetic center modulates the spin-spin interaction causing decay of the electron spin echo signal of the second center. The echo decay rate has a characteristic local maximum when the spin-spin interaction is comparable to $1/T_1$ of the fast-relaxing center and an amplitude comparable to the magnitude of the interaction. Use of this local maximum in echo decay rate to measure distance through the dipolar interaction can be quite attractive when at least one of the centers has a broad spectrum and limited excitation bandwidths make DEER problematic. Examples of distance measurement in hemeproteins will be shown.

When the two spins have isotropic *g*-factors, their interactions are conveniently described using a dipolar tensor and occasionally a scalar exchange interaction. The spin echo decay function is readily calculated using the properties of the dipolar tensor. However, when the spins do not have an isotropic *g*-factor, the spin-spin interaction is no longer a tensor making averaging of the decay function over orientational distributions much more tedious. It creates similar problems in the analysis of DEER and RIDME measurements. In such situations we have found it expedient to calculate the spectral broadening function and then recover the echo decay function from its Fourier conjugate.

Calculation of the spectral broadening function in this manner quickly reveals a singularity where the intensity goes to infinity. This singularity causes the echo decay function to asymptotically approach $\tau^{-1/2}$ at long times, which is not recognized from conventional calculations of the decay. This singularity is avoided when spin-lattice or self-relaxation of the *observed* paramagnetic center is included in the calculations.

This research is supported by the National Institutes of Health through GM069104 and HL095820.

Unveiling the Hsp90 ATPase cycle using nitroxide spin labelling and DEER spectroscopy reveals unknown dynamics of a molecular chaperone

Dipali Patel[*], Micha B. A. Kunze[*], Katharina F. Pirker[*], John E. Ladbury[*,**] and Christopher W.M. Kay[*]
[*]Institute of Structural and Molecular Biology, University College London, Gower Street, London WC1E 6BT, UK
[**]M.D. Anderson Cancer Research Centre, University of Texas, Houston, Texas, USA

Heat-shock protein 90 (Hsp90) is an extremely well studied, yet not fully understood, molecular chaperone. The vast number and diverse profile of its clientele include those that are involved in processes such as signal transduction and cell cycle regulation. The activation of these client proteins, which include oncogenic protein kinases, occurs via the binding and hydrolysis of ATP; this results from transient N-terminal domain dimerization.

This process and the resulting conformational changes thereafter have been extensively studied using techniques such as X-ray crystallography and FRET. It has been proposed that Hsp90 undergoes various conformational steps, mainly within the N-terminal and Middle domains of the dimer, to ultimately form the 'split' ATPase with 'closed and twisted' conformation [1,2].

Here we investigate the conformational stages of the Hsp90 ATP cycle using Double Electron-Electron Resonance (DEER) spectroscopy, in which the full length yeast Hsp90 dimer was labelled at specific positions with MTSSL. Distances between two nitroxide spin labels attached to a cysteine mutant on identical sites of both monomers of the dimer were measured in the nucleotide-free, AMP-PNP bound (non-hydrolysable analogue of ATP) and ATP-$_{\Upsilon}$S bound (slowly-hydrolysing ATP analogue).

Surprisingly, the results of our distance measurements revealed that the "closed and twisted' conformation of the ATP cycle may not be a true representative of the ATP-bound state of Hsp90. Contrary to observations made using FRET [3], the C-terminal domain of the dimer, which has been 'assumed' to be homo-dimerised, seems to open and close in both apo and ligand bound states, independent of N-domain dimerisation. Thus, our findings give a new perspective into the conformational cycle of Hsp90.

[1] Neckers, L., Tsutsumi, S., & Mollapour, M., Visualizing the twists and turns of a molecular chaperone. *Nat Struct Mol Biol* 16 (3), 235-236 (2009).

[2] Hessling, M., Richter, K., & Buchner, J., Dissection of the ATP-induced conformational cycle of the molecular chaperone Hsp90. *Nat Struct Mol Biol* 16 (3), 287-293 (2009).

[3] Ratzke, C., Mickler, M., Hellenkamp, B., Buchner, J., & Hugel, T., Dynamics of heat shock protein 90 C-terminal dimerization is an important part of its conformational cycle. *Proc. Natl. Acad. Sci. USA* 107 (37), 16101-16106 (2010).

Structure of the transcription elongation factor Spt4/5

Daniel Fielden[*], Dina Grohmann[*], Daniel Klose[**], Johann Klare[**], Heinz-Jürgen Steinhoff[**], Christopher W. M. Kay[*] and Finn Werner[*]

[*] Institute of Structural & Molecular Biology, University College London, Gower Street, London WC1E 6BT, UK.
[**]Department of Physics, University of Osnabrück, Barbarastrasse 7, 49076 Osnabrück, Germany

Spt4/5 is the only known universally conserved transcription factor, with homologues in all three domains of life. It enhances the processivity of RNA polymerase (RNAP) by enclosing the DNA entry channel, preventing template DNA dissociation. Spt5 contains two domains; a NusG N-terminal (NGN) domain and a C-terminal Kyrpidis, Ouzounis and Woese (KOW) domain (See Figure 1). Domain deletion studies have established that the NGN domain confers the processivity-enhancing effects of the protein. Spt4 interacts with the NGN domain of Spt5, providing a stabilising effect.

It has proved difficult to obtain complete structural information for Spt4/5. A crystal structure of the Spt5 NGN domain bound to Spt4 from the thermophilic arahaea *Methanococcus jannaschii* has been obtained and the KOW domain was subsequently modelled on [1]. A complete crystal structure of Spt4/5 from *Pyrococcus furiosus* has recently been published [2], although the structure may be affected by crystal packing effects.

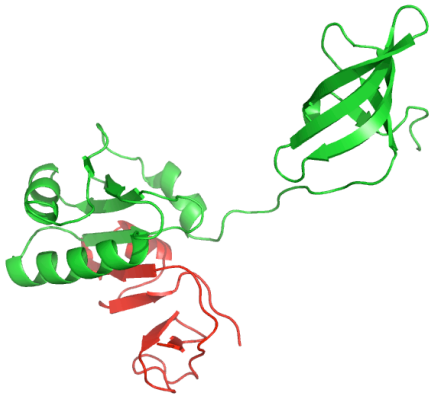


Figure 1. Model of Spt4/5. Spt4 (red) is shown bound to the NGN domain of Spt5 (green).

In order to validate current structural models of the NGN and KOW domains of Spt5, we have generated double cysteine mutants of Spt5, which we have coupled to 3(2-iodoacetamido)-PROXYL and are determining interdomain distances using DEER spectroscopy.

[1] Hirtreiter, A., Damsma, G. E., Cheung, A. M. C., Klose, D., Grohmann, D., Vojnic, E., Martin, A. C. R., Cramer, P. and Werner, F. (2010) Spt4/5 stimulates transcription elongation through the RNA polymerase clamp coiled-coil motif, *Nucleic Acids Res.*, **38** 4040-4051

[2] Klein, B. J., Bose, D., Baker, J. J., Yusoff, Z. M., Zhang, X., and Murakami, K. S. (2010) RNA polymerase and the transcription elongation factor Spt4/5 complex structure. *PNAS*, **108** 546-550

ELDOR study of the full-length Agp1 phytochrome from *Agrobacterium tumefaciens*

Sylwia Kacprzak[*], Anja Korn[*], Ibrahim Nijmona[**], Tilman Lamparter[**] and Stefan Weber[*]

[*]Institut für Physikalische Chemie, Albert-Ludwigs-Universität Freiburg
Albertstr. 21, 79104, Freiburg, Germany
[**]Karlsruhe Institute of Technology, Botanical Institute I, Kaiserstr. 2, 76131 Karlsruhe, Germany

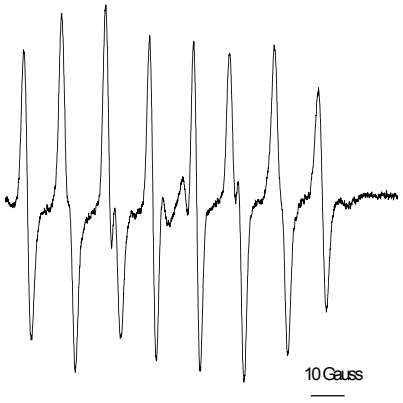
Phytochromes, photoreceptors found in plants, bacteria, and fungi, are most sensitive in the red (Pr-form) and far-red (Pfr-form) region of the visible spectrum. In plants they control a large number of developmental processes, such as seed germination, de-etiolation or flower induction. More than 50 years ago the first plant phytochrome was discovered, however, understanding of these photoreceptors has been stimulated only after the discovery of homologous microbial phytochromes – bacteriophytochromes. Typical bacterial phytochrome as Agp1 from *Agrobacterium tumefaciens* contains a C-terminal histidine kinase module, activity of which is modulated by the protein conformational changes induced in the N-terminal chromophore module. Up to now no crystal structure of full-length phytochrome of any kind is available. And the understanding of how do signals originating in the chromophore and its binding pocket propagate to the remote effector domain is missing.
Here we apply pulsed electron-electron double resonance (ELDOR) to investigate the spatial arrangement of the homodimer of the full-length Agp1 phytochrome from *A. tumefaciens* in its Pr form. Distances between two nitroxide spin labels attached at various positions are measured. These artificial paramagnetic probes, spin labels, are incorporated into the protein by site-directed spin labeling procedure via cysteine-substitution mutagenesis followed by the modification of the sulfhydryl groups with a nitroxide reagents like e.g.: MTSL ((1-oxyl-2,2,5,5-tetramethylpyrroline-3-methyl)-methanethiosulfonate).
Future measurements on the Agp1 in Pfr form will provide an insight into intramolecular signal transduction from chromophore to the effector domain.

Detection and characterization of superoxide radicals by using spin trapping method with DEPMPO

Laura Bolojan[*], V. Miclaus[**], G.Damian[*]

[*]Faculty of Physics, "Babes-Bolyai" University, Cluj-Napoca, Romania,
[**]Faculty of Chemistry and Chemical Engineering, "Babes-Bolyai" University, Cluj-Napoca, Romania

Reactive oxygen species (ROS), particularly the hydroxyl and superoxide radicals, are involved in many biochemical reactions and have been implicated in many adverse reactionare highly reactive chemicals. Therefore, the detection and characterization of $O_2^{\cdot -}$ in in vitro and in vivo systems has attracted considerable attention due to the important role that $O_2^{\cdot -}$ plays in cell signaling and immune response. The main difficulty in detection and characterization of reactive oxygen species, is given the short time of their life. As for free radicals, the only technique that can detect them directly is *Electron Paramagnetic Rresonance (EPR)* using spin trapping method. This technique, involves the indirect detection by addition of a primary free radical across the double bond of a diamagnetic compound named *spin trap* to form a more stable radical than the primary free radical named *spin aducts*[1,2]. In our paper we studied the detection and characterization of Potassium superoxide in alkaline solutions, which has been demonstrated to be a reliable source of superoxide, by EPR spectroscopy using DEPMPO spin trap. Higher stability of DEPMPO adducts yields a higher S/N ratio. In addition, ^{31}P (I = 1/2) induces extra line splitting, leading to more complex but also more informative spectra when comparing with other spin trap [3].



EPR spectrum of DEPMPO-superoxide spin aduct

References:

[1] R.V.Lloyd, P.M. Hanna, R.P. Mason, Free Radical Biology & Medicine, 22, 885-888, 1997
[2] G.R.Buettner, R.P.Mason, Methods Enzymol, 1990; 186:127– 33, 1990
[3] K. Stolze, N. Udilova, H. Nohl, Free Radical Biology & Medicine, Vol. 29, No. 10, pp. 1005–1014, 2000

Pulsed EPR investigation of Cu^{2+} metal ions in novel copper containing Metal Organic Frameworks (MOFs) .

Chandrima Pal*, M. I. M. Mohideen**, H. ELMkami#, R. E. Morris**, O. Schiemann*

*Biomolecular Sciences Research Complex,**EaSTChem School of Chemistry, #School of Physics and Astronony, University of St-Andrews, UK.

Metal-organic frameworks (MOFs) compounds have attracted a considerable attention and been widely investigated for applications in storage, separation and catalysis. MOFs are crystalline nano-porous materials composed of two basic building units, transition metal ions and organic linkers. The appropriate design of ligands and metal building units have led to various interesting porous MOFs but the control of the shape and the properties of their formed cavities is still challenging. In the present work the metal ion Cu^{2+}, which is part of our MOFs, is used as paramagnetic probe to obtain additional information and to gain further insight into the structural properties of a novel copper containing MOF.

Interaction between methemoglobin and bioactive glasses studied by EPR spectroscopy

C. Gruian[*,**], J. P. Klare[*], S. Boehme[*], S. Simon[**], H. J. Steinhoff[*]

[*]Department of Physics, University of Osnabrueck, 49069 Osnabrueck, Germany
[**] Faculty of Physics, Babes-Bolyai University, 400084, Cluj-Napoca, Romania

Although functional protein adsorption at liquid-solid surfaces has been intensively studied in the last years, a detailed knowledge of the accompanying conformational changes upon protein adsorption is still elusive. In this study we applied combined SDSL and EPR spectroscopy [1] to investigate the adsorption of two model proteins, horse methemoglobin and MnmE from *E. coli*, on bioactive glasses which are commonly used as biomaterials for bone repairing. Prior protein functionalization, the surface of bioactive glasses was modified using 3-aminopropyl-triethoxysilane and glutaraldehyde. In order to extract structural information, EPR spectra of the proteins in solution were compared to those recorded after adsorption.

Horse methemoglobin containing cysteine at the positions β-93 was spin-labeled with iodoacetamid spin label. In case of MnmE, the positions mutated to cysteine for spin labelling with methanethiosulfonate spin label are situated in the G-domain and in the N terminal of the protein, to provide information on the position of the G domains relative to each other in both open (apo) and closed (transition) state [2], when the protein is adsorbed on the bioactive glass.

In order to gain detailed information about the binding and the immobilization of the two proteins upon the adsorption onto bioglass, we performed room temperature cw EPR measurements. Four-pulse DEER spectroscopy [3] was then used to test whether the structure in close vicinity to the chosen positions suffers any changes or if the protein keeps its native conformation upon adsorption. The cw-EPR spectra revealed that the fraction of immobilized spin labels is increasing in the adsorbed state while the low modulation depth characteristic for all the DEER traces recorded in adsorbed state suggest that dissociation of the proteins is possible to occur during adsorption.

The authors wish to thank for the financial support provided from programs co-financed by The SECTORAL OPERATIONAL PROGRAMME HUMAN RESOURCES DEVELOPMENT, Contract **POSDRU 6/1.5/S/3 – „**Doctoral studies: through science towards society**".**

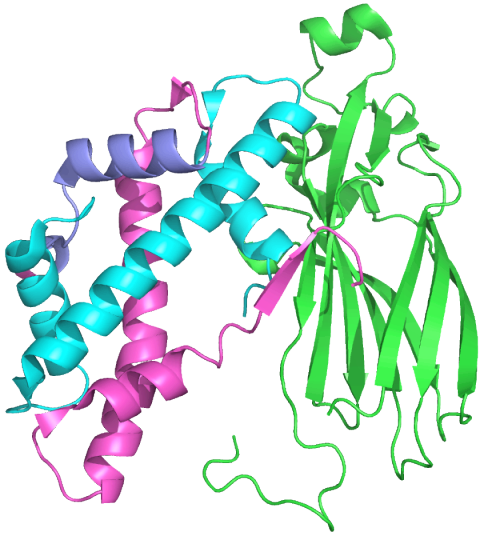
[1] J. P. Klare, H.J. Steinhoff, *Photosynth Res*, 102, 377–390, **2009**
[2] S. Meyer, S. Boehme, A. Krueger, H.J. Steinhoff, J.P. Klare, A. Wittinghofer-*PLoS Biol*, 7 (10), **2009**
[3] J.P. Klare, H.J. Steinhoff, "*Site-directed Spin Labeling and Pulsed Dipolar Electron Paramagnetic Resonance",* Encyclopedia of Analytical Chemistry, **2010**.

PELDOR spectroscopy used to study histone chaperones

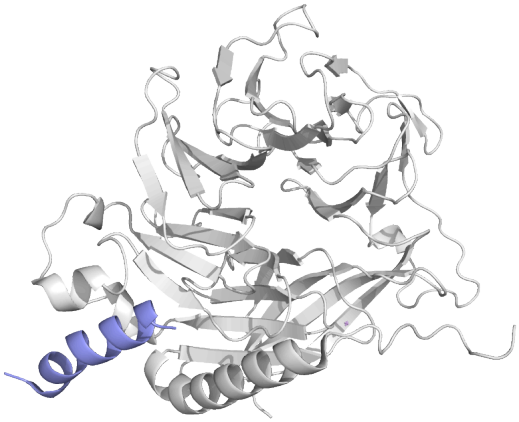
R.Ward[1], A.Bowman[2], M. Tyl[3], T. Owen Hughes[2], E. Laue[3], D. Norman[2]

[1]School of Biology, University of St Andrews, KY16 9ST
[2]College of Life Sciences, University of Dundee, Dundee, DD1 5EH
[3]Department of Biochemistry, University of Cambridge, Cambridge, CB2 1GA

Histone proteins (H2A, H2B, H3 and H4) associate to form an octameric protein particle (two H2A-H2B dimers and one H3-H4 tetramer) that binds, stabilises and compacts genomic DNA into nucleosomes, eventually leading to the formation of higher order structures such as chromosomes. During processes such as transcription and translation access to the DNA is required, this is achieved by removing histones from the octameric particle. Histone chaperones are known to assist in both nucleosome assembly and disassembly via interaction with the histone proteins, however the molecular details are still not clear for all the members of this class of protein. Asf1 is a histone chaperone that is known to disrupt the H3-H4 tetramer to form an Asf1-(H3-H4 dimer) complex [1]. RbAp48 is another histone chaperone that is known to bind to H3 and H4 [2], but there is little structural data on these interactions. We used site-specific labelling and PELDOR spectroscopy [3] to characterise the binding interactions of the H3-H4 tetramer with both Asf1 and RbAp48. By comparing the results we were able to draw conclusions about the alternative binding interactions that occur between each histone chaperone and the H3/H4 proteins.



Asf1 (green) –H3 (light blue)-H4 (magenta/dark blue)



RbAp48 (grey) –H4 fragment (dark blue)

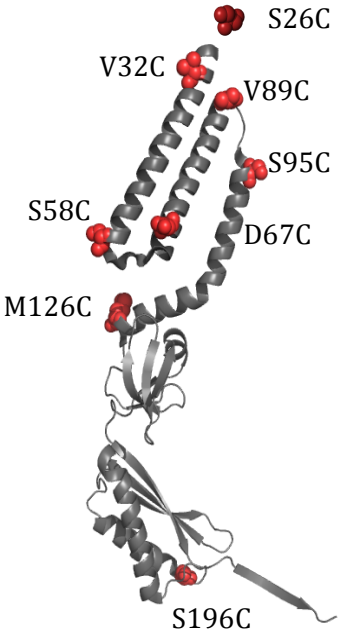
[1] English CM, et al. Cell. 2006 Nov 3;127(3):495-508.
[2] Murzina NV, et al. Structure. 2008 Jul;16(7):1077-85.
[3] Milov AD, Salikohov KM, Shirov MD. Fiz. Tverd. Tela 1981, 23, 975 – 982.

Investigation of the Mechanosensitive Channel of Small Conductance (MscS) by PELDOR Spectroscopy

Christos Pliotas[a], Emma Branigan[a], Richard Ward[a], Akiko Rasmussen[b], Ian R. Booth[b], Olav Schiemann[a], and James H. Naismith[a]

[a]Biomolecular Sciences Research Complex, University of St Andrews, North Haugh, St Andrews, KY16 9ST, UK
[b]Institute of Medical Sciences, University of Aberdeen, Foresterhill, Aberdeen, AB25 2ZD, UK

The mechanosensitive channel of small conductance (MscS) acts as a pressure safety valve that opens in response to membrane tension to prevent cell lysis. MscS is a homoheptameric integral inner-membrane protein that has been characterized by two crystallographic studies, depicting two discrete conformational states. However, it remains unclear how the channel gates. Here, we report our progress on applying Pulsed Electron-Electron Double Resonance (PELDOR) spectroscopy on MscS. We emphasize on challenges in ensuring complete labeling of the mutants and sample reproducibility.



Single-cysteine functional mutants of MscS have been purified and MTSL-labeled, spanning all three transmembrane helices, which are crucial for gating, TM1, TM2 and TM3. S26C is in the non-resolved short periplasmic domain of TM1. V32C and S58C are on the top and bottom of TM1, respectively. D67C is on the bottom of TM2 and V89C at the top of TM2 next to the loop between the two helices. S95C is in the middle of $TM3_a$ within the channel pore and M126C is on the edge of $TM3_b$, at the interface between membrane and cytoplasm. S196C is on the large cytosolic domain, which forms a vestibule of unknown function. These distance measurements will contribute towards the elucidation of conformational states of MscS. This research project is supported by the BBSRC.

Monomer of MscS (grey)-Spin labelled residues (red)

[1] Bass RB, et al. Science. 2002; 298(5598): 1582-7.
[2] Wang W, et al. Science. 2008; 321(5893): 1179-83.
[3] Hagelueken G, et al. Angewandte Chemie (International ed. in English). 2009; 48(16): 2904-6.

UVA-induced radical damage in human fibroblasts, pigmented melanocytes, and cell organelles: dissecting the different radicals within each system using ESR/spin trapping

N Kassouf[1], Mark Birch-Machin[2] and R. M. Haywood[1]

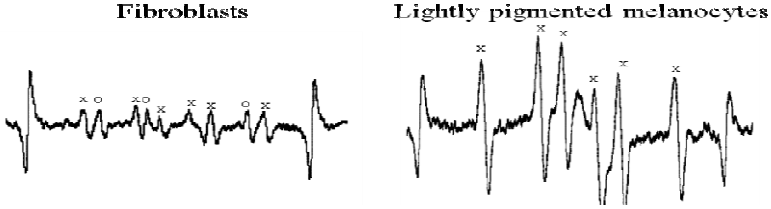
[1] RAFT Institute, Mount Vernon Hospital, Northwood, Middlesex, HA6 2RN
[2]Department of Dermatological Sciences, Institute of Cellular Medicine, Medical School, Newcastle University, Newcastle upon Tyne NE2 4HH, UK.

UVA irradiation from sunlight has been implicated in causing melanoma, and it is hypothesised that it may derive from a melanin-dependent mechanism. We aim to determine the cellular and sub-cellular consequences of UVA exposure in pigmented and non-pigmented cells using ESR/spin trapping in order to establish the specific types of free radical damage that occur. We also aim to understand whether there may be a pathophysiological role for melanin in exacerbating UVA-induced cell damage.

CW-ESR was used to analyse free radical damage in normal human skin fibroblasts and lightly-pigmented human epidermal melanocytes (HEMa-LP) using the spin trap 5,5 dimethyl-1-pyrroline *N*-oxide (DMPO). When cells were exposed to UVA irradiation, carbon-centred radicals were detected in both normal cell types that were consistent with those previously detected in melanoma cell lines. Carbon-centred radical-adducts (x) were greater in intensity in HEMa-LP compared to skin fibroblasts, however we also detected adducts assigned to secondary alkoxyl radicals (o) in fibroblasts [1], that are absent in HEMa-LP cells (Fig.1). DMPO carbon-adducts were simulated using hyperfine splittings a(H) = 2.3mT and a(N) = 1.59mT, and comparable to irradiated genomic DNA/melanin and salmon sperm DNA/melanin. The DMPO oxygen-adduct was simulated using a(N) = 1.6mT and a(H) = 1.45mT. In addition, within the HEMa-LP spectrum we detected an underlying stable radical as yet unassigned but proposed to be a DNA radical [1]. Non-pigmented melanoma cell mitochondria and nuclei containing DMPO were also UVA-irradiated. Weak carbon- and oxygen-centred radical-adducts were detected in mitochondria and comparable to those in skin fibroblast cells, and a protein radical-adduct was significant at high modulation. In contrast, we detected a stable radical in irradiated nuclei proposed to be a DNA radical (see abstract R Haywood).

We have demonstrated for the first time, UVA-induced free radicals in normal skin cells using ESR. The carbon-centred radicals trapped with DMPO in whole cells and which we propose to be DNA radicals may be involved in the pathophysiological changes associated with skin carcinogenesis and melanoma in pigmented cells.

Fig.1



[1] Haywood, R., Andrady, C., Kassouf, N. and Sheppard N. *Photochem. Photobiol.* (2011) 87(1), 117-130.

Pitfalls and progress in measuring the orientational dependence of DEER for transition metals in model systems and proteins.

A. M. Bowen*, M. W. Jones*, J. E. Lovett*, J. Harmer*, J. R. Dilworth* and C. R. Timmel*

*Centre for Advanced Electron Spin Resonance, University of Oxford, Inorganic Chemistry Department, South Parks Road, Oxford, OX1 3QR.

This work presents a complete study, including measurement and analysis, of the orientational selectivity in DEER spectra recorded between two copper(II) centres in the protein homodimer of copper amine oxidase from *Arthrobacter globiformis* as well as model chemical systems with differing inter-copper distances. Spectra were recorded at X-band on a commercial Brucker Elexys spectrometer and simulated spectra were fitted to the experimental data using a least-squares algorithm.

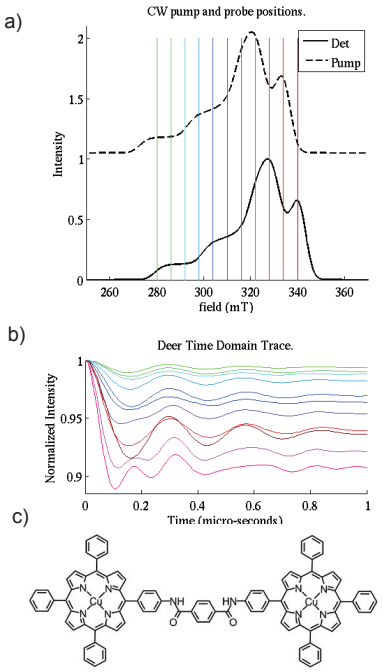


Fig. 1 a) DEER Pump and probe positions across the copper spectrum, for a di-copper model system, with a 200 MHz separation. b) Resultant simulated DEER spectra showing orientational selectivity. c) Structure of the di-copper model system.

The degree to which orientational selectivity affects the shape of the resultant DEER trace differs across the EPR spectrum with regard to the selected pump and probe positions (*Fig. 1*). Therefore in order to observe significant orientational effects some prior knowledge of the system is required.

Orientationally selective DEER spectra were simulated using a home-written software in Matlab.[1] This program requires an initial structural input; in the case of the copper amine oxidase this was an X-ray structure of the protein. However for the model systems it was necessary to use a combination of Density Functional Theory (DFT) calculations and X-ray structures of chemical precursors to predict likely conformers of the molecular structure. In order to verify the structural model used for the model systems similar Copper-Nitroxide and Nitroxide-Nitroxide compounds were also studied. The bi-nitroxide systems were further analysed using DEERAnalysis.[2] However such analysis is not correct when significant orientational selection is present. DFT calculations were also used to confirm the orientation of the g-matrix with respect to the molecular structure for both the model systems and the protein.

[1] J. E. Lovett, A. M. Bowen, *et al., Physical Chemistry Chemical Physics*, 2009, **11**, 6840-6848.

[2] G. Jeschke, V. Chechik, *et al., Applied Magnetic Resonance*, 2006, **30**, 473-498.

Room temperature ionic liquids: Pulse EPR Spectroscopy used to unravel solution structures

Daniel Kattnig, Yasar Akdogan, Jeannine Heller, and Dariush Hinderberger

Max Planck Institute for Polymer Research, Ackermannweg 10, D-55128 Mainz.

Room temperature ionic liquids (RTIL) have emerged as intriguing reaction media in chemical engineering, in particular in the context of green chemistry.[1] Their application spectrum rests upon their negligible vapour pressure, their amphiphilicity, and the fact that novel chemical reaction are being discovered that can only be carried out in these solvents.[2,3]
The spin probe methodology has fruitfully been applied to study RTILs by EPR spectroscopy.[4-7] Several studies have focused on the rotational tumbling or the isotropic nitrogen hyperfine coupling constant of the probe making micro-viscosities and polarities experimentally accessible. Despite these efforts, the immediate surroundings of the probe and the nature of specific probe/solvent interaction have remained elusive.
We have employed several strategies to unravel the details of the solvent structure in RTILs: High-field pulsed EPR spectroscopy at W-band frequencies has been employed to unequivocally determine hyperfine interaction tensors and g-matrices. g_{xx} and A_{zz} of several TEMPO-based spin probes in RTILs of the 1-n-alkyl-3-methyl imidazolium type have been analysed. Polarity-proticity plots reveal the interaction of the nitroxide moiety with apolar and ionic nanodomains, depending on the probe structure.[8] We have studied the solvation of potassium nitrosodisulfonate (Fremy's salt) in 1-buthyl-3-methyl imidazolium tetrafluoroborate (bmimBF_4) by means of high field, pulse (Mims- and Davies-type) ENDOR spectroscopy. By orientation-selective measurements we attempt to directly observe the hyperfine interaction tensors associated with the protons of the solvent cation. In combination with molecular dynamics simulations a detailed picture of the spin probe surroundings can be reconstructed. For the first time we also report on the investigation of binary water/RTIL mixtures by means of EPR spectroscopy. The hydrophilic bmimBF_4 forms nanostructures when diluted with water.[9] The nanostructure formation is evidenced by analysis of the rotational correlation times and the isotropic nitrogen hyperfine interaction constant of TEMPOL doped into the solution. HYSCORE spectra of TEMPOL-d_{17} in mixtures of D_2O/bmimBF_4 can be used to assess the surroundings of the spin probe.

[1] Plechkova, N. V.; Seddon, K. R.; *Chem. Soc. Rev*. **2008**, *37*, 123-150.
[2] Wasserscheid, P., Welton, T.; Ionic Liquids in Synthesis: Wiley-VCH Weinheim, Germany, 2003.
[3] Ohno, H.; Electrochemical Aspects of Ionic Liquids: Wiley – Interscience: Hoboken, NJ, 2005.
[4] Evans, R. G.; et al.; *ChemPhysChem*, **2005**, 6(6), 1035-1039.
[5] Strehmel, V.; et al.; *J. Phys. Org. Chem*. **2006**, *19(5)*, 318-325.
[6] Strehmel, V.; et al..; *ChemPhysChem*, **2008**, *9(9)*, 1294-1302.
[7] Kawai, A.; Hidemori, T.; Shibuya, K.; *Chem. Let*. **2004**, *33(11)*, 1464-1465.
[8] Akdogan, Y., Heller, J., Zimmermann, H.; Hinderberger D.; PCCP 2010, 12, 7874–7882.

Electron Transfer in proteins of the photolyase/cryptochrome family: Light-induced radical-pair formation is more complex than initially anticipated

Till Biskup,[*,†] Asako Okafuji,[*] Kenichi Hitomi,[**] Elizabeth D. Getzoff,[**] Gebhard Kaiser,[***] Alfred Batschauer,[***] Adelbert Bacher,[****] Erik Schleicher,[*] and Stefan Weber[*]

[*]Institut für Physikalische Chemie, Albert-Ludwigs-Universität Freiburg, Freiburg, Germany
[**]Department of Molecular Biology and The Skaggs Institute of Chemical Biology, The Scripps Research Institute, LaJolla, U.S.A.
[***]Fachbereich Biologie, Philipps-Universität Marburg, Marburg, Germany
[****]Lehrstuhl für Organische Chemie und Biochemie, Technische Universität München, Garching, Germany
[†]Current address: Physical and Theoretical Chemistry Laboratory, University of Oxford, Oxford, United Kingdom

A conserved triad of tryptophan (Trp) residues in the photolyase/cryptochrome family facilitates light-induced electron transfer (ET) from exogenous reductants at the protein surface to the flavin adenine dinucleotide (FAD) cofactor in the protein core. The highly conserved positions and orientations of the three Trps in all structure-known members of the photolyase/cryptochrome family suggest that ET along this path is relevant. Indeed, FAD photoreduction has been consistently observed among photolyases and cryptochromes from different organisms regardless of the proteins' functions [1–3].

In this contribution we present EPR and UV/vis results from cryptochromes of the DASH-type and class-II photolyases of plants. Amino-acid alignments of the latter proteins suggest that the conserved Trp triad is missing. Surprisingly, photoreduction kinetics of class-II enzymes are nevertheless very similar to those of the other classes with even higher photoreduction rates in class II as compared to class I.

Furthermore, in the cryptochromes of *Xenopus laevis* and *Synechocystis* sp. PCC6803 we discovered variable electron-transfer pathways that are differentially used in these proteins despite their high degree of structural conservation. Taken together, these observations demonstrate, that ET and radical-pair formation in proteins of the photolyase/cryptochrome family are considerably more complex that commonly assumed.

[1] T. Biskup, E. Schleicher, A. Okafuji, S. Weber, K. Hitomi, E.D. Getzoff, S. Weber, *Angew. Chem. Int. Ed.* 48 (2009) 404–407

[2] S. Weber, *Biochim. Biophys. Acta – Bioenergetics* 1707 (2005) 1–23

[3] S. Weber, C.W.M. Kay, H. Mögling, K. Möbius, K. Hitomi, E.D. Getzoff, *Proc. Natl. Acad. Sci. U.S.A.* 99 (2002) 1319–1322

[4] A. Okafuji, T. Biskup, K. Hitomi, E.D. Getzoff, G. Kaiser, A. Batschauer, A. Bacher, J. Hidema, M. Teranishi, K. Yamamoto, E. Schleicher, S. Weber, *DNA Repair* 9 (2010) 495-505

Using EPR up close and from afar: elucidating mechanisms in haem copper oxidases

Fraser MacMillan[1], Jessica van Wonderen[1], Myles Cheesman[1], Iris van der Hocht[2], Heike Angerer[3], Robert Gennis[4], & Hartmut Michel[2]

[1] University of East Anglia, Henry Wellcome Unit for Biological EPR, Norwich, U.K.; [2]Max-Planck-Institut für Biophysik, Frankfurt am Main, Germany; [3] J. W. Goethe-Universität Frankfurt, Zentrum der Biologischen Chemie, Frankfurt am Main, Germany; [4] University of illinois at Urbana-Champaign, Department of Biochemistry, Urbana, U.S.A.

Haem copper oxidases constitute the terminal complex of the respiratory chain and catalyse the reduction of oxygen to water. This exergonic redox reaction is coupled to proton pumping across the inner mitochondrial or bacterial membrane. O_2 reduction occurs at the binuclear haem-Cu_B centre. Despite high resolution X-ray crystallographic structures, the properties of the catalytic redox states of the metal centres and their relation to protonation states within this class of enzyme remain still poorly understood [1]. Modern EPR techniques (also in combination with magneto-optical studies) enable us to probe different catalytic intermediate states either directly or indirectly [2,3].

From afar pulsed ELDOR spectroscopy, a technique for accurately measuring inter spin distances in the range 2-8 nm, is used to resolve subtle structural changes when applied to spin-labelled systems trapped in different intermediate states (e.g. P, R & F states) and which allows the study of local conformational changes in great detail. Using this technique conformational change within the proton uptake channels is discussed.

Up close both EPR and magneto-optical techniques (Magnetic Circular Dichroism) are used to address the nature of the metal ligands in the binuclear centre as well as transiently formed radical species from different intermediate states as well as in oxidases from different species [1,3].

This research is supported by the Max-Planck Society and the Royal Society. Fraser MacMillan is holder of a Royal Society Wolfson Research Merit Award.

[1] I. van der Hocht, J. van Wonderen, F. Hilbers, H. Angerer, F. MacMillan, & H. Michel, Interconversions of P and F Intermediates of Cytochrome *c* Oxidase from Paracoccus denitrificans *Proc. Natl. Acad. Sci. USA*, in press (2011)

[2] H. Käß, F. MacMillan, B. Ludwig, T. F. Prisner Investigation of the Mn binding site in cytochrome *c* oxidase of *Paracoccus denitrificans* by multi-frequency EPR *Journal of Physical Chemistry B* 104, 5362-5371 (2000)

[3] Fraser MacMillan, Aimo Kannt, Julia Behr, Thomas F. Prisner & Hartmut Michel Direct evidence for a tyrosine radical in the reaction of cytochrome *c* oxidase with hydrogen peroxide *Biochemistry* (accelerated) 38, 9179-9184 (1999)

Conformational Changes Of Vinculin Tail Upon Binding To F-Actin And Phospholipid Membranes Studied By EPR Spectroscopy

Christoph Abé[*], Franziska Dietrich[**], Wolfgang Ziegler[**], Heinz-Jürgen Steinhoff[*]

[*]Physics Department, University Osnabrueck, Germany.
[**]IZKF Leipzig, Germany.

Embryogenesis, tissue regeneration, wound healing and immune responses are based on cell adhesion and migration events. The ability of cells to link the actin cytoskeleton to cell adhesion protein receptors is essential for these processes in which the cytoskeletal protein vinculin plays a key role [1]. Vinculin consists of a globular head and a tail domain (Vt). Binding of Vt to F-actin enables the connection of the cytoskeleton to the transmembrane receptors and stabilization of focal adhesion sites. Moreover, acidic phospholipids, as part of the cell membranes, play an essential role in the regulation of cell migration. Understanding of the underlying molecular interactions requires the knowledge of the conformations of Vt under physiological conditions in the absence and presence of the above mentioned binging partners. CW and pulse EPR methods (DEER and orientation selection DEER) [2, 3] are used in the present work to study spin labeled Vt in solution and to follow its structural changes induced upon F-actin and acidic phospholipid binding. The EPR results in combination with a rotamer library approach [4], MD simulations [5] and a grid search algorithm allowed for modeling of monomeric and dimeric Vt in the presence and absence of binding partners. Inter-spin distance data show that the five helix bundle configuration found in the crystal structure is largely preserved in solution and a model for the dimeric conformation is obtained. The results for Vt bound to F-actin unravel that the bundle domains spatially expand. Upon interaction of Vt with acidic phospholipid vesicles the bundle opens and binds to the surface of the membrane bilayer. Inter-spin distance data and the analyses of the mobility and the accessibility of the spin label side chains allowed for modeling of the Vt conformations in solution, bound to F-actin or to the phospholipid bilayer surface. In cell migration Vt is supposed to continuously undergo transitions between these three conformational states.

[1] Ziegler, W. H., R. C. Liddington, and D. R. Critchley. *Trends in Cell Biology* 16:453-460, **2006**.
[2] Bordignon, E. and H.-J. Steinhoff. *In* Biological Magnetic Resonance 27 - ESR Spectroscopy in Membrane Biophysics. M. A. Hemminga and L. J. Berliner, editors. Springer Science and Business Media, New York. 129-164, **2007**.
[3] Jeschke, G. and Y. Polyhach. *Physical Chemistry Chemical Physics* 9:1895-1910, **2007**.
[4] Polyhach, Y., E. Bordignon, G. Jeschke. *Physical Chemistry Chemical Physics* 13:2356-2366, **2011.**
[5] Beier, C. and H.-J. Steinhoff. *Biophys. J.* 91:2647-2664, **2006**.

Investigation of the molecular mechanisms underlying ks-vFlip activation of the IKK Complex by probing the IKKγ transition using site directed spin-labeling and pulsed EPR

Claire Bagnéris*, Kacper B. Rogala**, Vlad Zamfir**, Selina Dagless**, Katharina F. Pirker**, Micha B. A. Kunze**, Tracey Barrett* and Christopher W. M. Kay**

* Institute of Structural & Molecular Biology, University College London, Gower Street, London WC1E 6BT, UK.
** Institute of Structural & Molecular Biology, Birkbeck College, University of London, London WC1E 7HX, UK.

The NF-κB pathway is a prime target for lymphogenic viruses that hijack the mechanisms for proliferation through the production of host mimetic viral proteins that cannot be regulated by the existing cellular mechanisms. One such virus is Kaposi's sarcoma herpes virus (KSHV), the main causal agent of Kaposi Sarcoma (KS) which occurs most frequently in HIV-infected individuals. Because in this particular case both crystallographic and NMR approaches are limited: IKKγ, the essential modulator of the NF-κB pathway, is thought to be a long parallel dimeric intermolecular coiled-coil, we use site-directed spin labelling in combination with EPR spectroscopy in order to gain insights on the mechanism of the IKKγ activation by ks-vFLIP.

The crystal structure of vFLIP bound to IKKγ [1] gave insights into the molecular basis underlying the interaction, but was unable to address the important questions of the conformation of IKKγ in its ground and activated states. In order to address this, nitroxide spin labels are attached to various positions in the dimeric IKKγ protein and distances between them are investigated by continuous wave (cw) EPR and Double Electron-Electron Resonance (DEER) in both the native protein and mutants responsible for disease. The resulting distance information is used to complement the known crystal structures, unveiling the spatial conformation of the full-length protein, which has so far eluded X-ray crystallography.

[1] Bagnéris C, Ageichik AV, Cronin N, Wallace B, Collins M, Boshoff C, Waksman G, Barrett T. Crystal Structure of a vFlip-IKKγ Complex: Insights into Viral Activation of the IKK Signalosome. Molecular Cell 2008; 30: 620-631.

Destination State Screening of Active Spaces in ESR Simulations

M. Krzystyniak, Luke J. Edwards*, Ilya Kuprov

Oxford e-Research Centre, University of Oxford, 7 Keble Road, Oxford OX1 3QG, UK.

luke.edwards@chem.ox.ac.uk

Abstract

The exponential scaling of matrix dimension with spin system size severely limits current simulation capabilities. Polynomially scaling algorithms are however made possible using various state space restriction techniques such as interaction graph analysis,[1] symmetry pruning,[2] zero track elimination,[3] and Liouvillian path tracing.[4]

Further improvement of these methods may be achieved by observing that only those spin system trajectories that pass through the detection state give rise to observed signal. Propagator orbits generated from the detection state are thus the only orbits that need to be simulated, leading in many cases to a large decrease in the computational effort required.

This procedure, *Destination State Screening*, can be shown to be formally exact, and may be applied on top of existing state space restriction techniques.[5] This screening procedure has been implemented in the latest version of the *Spinach* software library.[6] Examples are given of the reductions in matrix dimension that are achieved compared to the original 'Source state screening' upon application of this technique. The table below gives a sample of the reductions observed in the simulation of liquid state ESR spectra.

Radical and symmetry	Full state space dimension	Initial basis set	Basis dimension	A_{1g} irrep. dimension	Source state screening	Destination state screening
methyl, S_3.	256	complete	256	80	1×1, 2×4.	1×4
phenyl, $S_2 \otimes S_2$.	4096	complete basis on electron,	128	72	3×1, 2×18.	1×18
chrysene, $S_2 \otimes S_2 \otimes S_2 \otimes S_2 \otimes S_2 \otimes S_2$.	6×10^7	zz terms on nuclei.	16384	2916	6×1, 2×729.	1×729

[1] I. Kuprov, N. Wagner-Rundell, P.J. Hore, *J. Magn. Reson.*, 189 (2007) 241-250.

[2] I. Kuprov, *J. Magn. Reson.*, In press, doi:10.1016/j.jmr.2010.12.004

[3] I. Kuprov, *J. Magn. Reson.*, 195 (2008) 45-51.

[4] H.J. Hogben, P.J. Hore, I. Kuprov, *J. Chem. Phys.*, 132 (2010) 174101.

[5] M. Krzystyniak, L.J. Edwards, I. Kuprov, *J. Magn. Reson.*, (Submitted manuscript).

[6] http://spindynamics.org

Development of an electron spin-based biosensor

Vishal A. Sanchania*,**, Katharina F. Pirker*, Micha B. A. Kunze*,
Felix Schumacher*,***, Sandrine Heutz****, John Ward*, Gabriel Aeppli** and
Christopher W. M. Kay*,**

* Institute of Structural & Molecular Biology, University College London, Gower Street, London WC1E 6BT, UK.
** London Centre for Nanotechnology, University College London, 17-19 Gordon Street, London WC1H 0AH, UK.
*** Department of Chemistry, University College London, 20 Gordon Street, London WC1H 0AJ, UK.
**** London Centre for Nanotechnology and Department of Materials and, Imperial College London, London, SW7 2AZ, UK.

Spectroscopic techniques are continually being adapted for use as sensors particularly by bio-functionalization; and EPR (Electron Paramagnetic Resonance) is no stranger to this approach. The early 1970s saw President Nixon's war on drugs, resulting in the deployment of the Free Radical Assay Technique or FRAT, a large scale EPR-based biosensor in Vietnam [1]. We aim to build upon the biosensing applications of EPR (including spintronic materials). Furthermore, by employing the use of site-directed spin labelling (SDSL) by labelling antigens and antibodies we hope to ultimately use EPR as a diagnostic immunoassay tool.

A 36kDa E.coli Ras like GTPase (ERA) protein has been engineered to encode two epitopes, an N-terminal poly-histidine and a C-terminal FLAG tag. Antibodies are commercially available to both epitopes. Through the tactical positioning of cysteine residues within the vicinity of these epitopes, we have shown changes in EPR lineshape of CW spectra upon addition of the respective antibodies. A FLAG peptide has also been spin labelled to demonstrate canonical antibody-antigen binding as proof of principle with future competition immunoassay potential.

[1] Edwin F. Ullman, *"Homogeneous Immunoassays: Historical Perspective and Future Promise"*, Journal of Chemical Education. 1999

Graphical user interface for the specification and visualization of interactions in large spin systems

G.T.P. Charnock, Ilya Kuprov
Oxford e-Research Centre, University of Oxford

We present a completed graphical user interface supporting the SpinXML standard for the specification and visualisation of interactions in large spin systems.

SpinXML is an XML based file format capable of storing the details of the spins and interactions of a spin system along with a set of nested reference frames in which these spins and interactions may be specified. Interactions can be specified in any one of five different conventions: scalar; matrix; eigenvalues and an orientation; axiality, rhombicity and an orientation; span, skew and an orientation. Orientations themselves may be specified as a directional cosine matrix, a set of Euler angles, a quaternion or an axis and an angle.

In addition to the SpinXML import/export filters, filters for several common file formats such as Gaussian and EasySpin are available. Additional import/export filters may be easily added GUI via a standard function interface. Full support for the use of nested reference frames has now been completed, making SpinXML a fully implemented standard.

The GUI runs on Linux and Windows workstations – a demonstration will be available at the poster presentation.

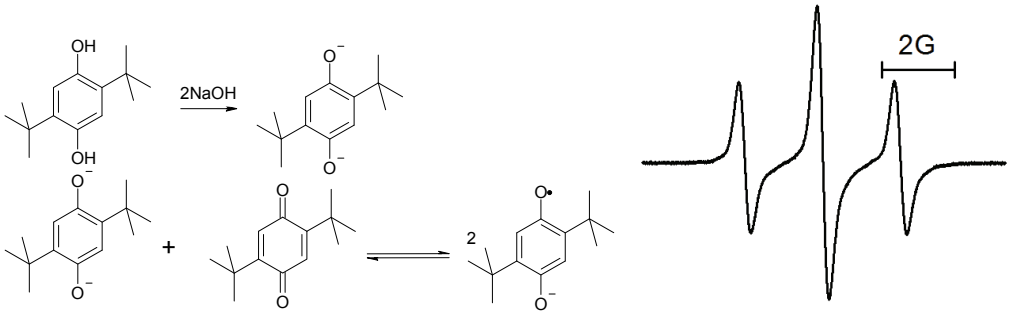
Comproportionation/disproportionation equilibrium between 3,5-di-tert-butyl-p-benzoquinone and the corresponding hydroquinone

Thomas Newby[*], Peter Price[**], Colin Lyons[**], Victor Chechik[*]

[*]University of York, Heslington YO10 5DD
[**] Nufarm Limited, Wyke Lane, Wyke, Bradford, BD129EJ

The redox chemistry of quinones and hydroquinones is exploited in nature and used in a number of chemical processes [1, 2]. For example, in the production of monomers, these compounds are used to stop spontaneous polymerisation. Therefore, understanding their general behaviour, such as the comproportionation / disproportionation equilibrium, will lead to optimising their use in these processes.

The comproportionation/disproportionation equilibrium described below is suited to be studied by EPR [1, 3]. We will demonstrate the change in the equilibrium of going from polar to apolar solvents. We will describe the fate of the different species in the equilibrium upon degradation, through a combination of EPR and Mass Spectrometry. We will also show the sensitivity of these compounds to slight variation in the experiment's parameters, such as the concentration of base.



[1] V Roginsky, L Pisarenko, W Bors and C Michel, *J. Chem. Soc.-Perkin Trans. 2*, 1999, 871-876.
[2] J Pedersen, *J. Chem. Soc.-Perkin Trans. 2*, 1973, 424-431
[3] A Kabadya, Z Luz and D Goldfarb, *J. Am. Chem. Soc.,* 1994, 5805-5813

Linked Antiferromagnetic Rings studied by Multi-frequency EPR Spectroscopy

Floriana Tuna, Grigore Timco, Thomas Faust, David Collison, Richard E. P. Winpenny, Eric J. L. McInnes

School of Chemistry, The University of Manchester, Oxford Road, M13 9PL, UK.

We became interested in linking anti-ferromagnetically coupled wheels because it has been found that they have potential for quantum information processing (QIP). For example, we have recently found that a pair of weakly coupled Cr_7Ni wheels (S = ½) is a promising candidate for a two-Qubit gate [1], while two Cr_7Ni wheels attached to a Cu(II) ion match the requirements needed to implement a three-qubit scheme [2]. If QIP is to be implemented, it is very likely we will have to meet very strict criteria for the exchange interaction between such molecular wheels.

Here we show that coordination chemistry offers us the flexibility to finely tune the magnetic exchange coupling between two or more polymetallic wheels. We also show that electron paramagnetic resonance (EPR) spectroscopy is a powerful technique, helping us to understand the electronic spin structure of these compounds and to quantify the interaction between wheels with a high degree of accuracy.

The results of multi-frequency (3 - 94 GHz) EPR studies on two different families of AF Cr_7Ni wheels will be presented. Magnetic communication between two, three or four prototype qubits is also probed by EPR spectroscopy.

1. A. Candini, G. Lorusso, F. Troiani, A. Ghirri, S. Caretta, P. Santini, G. Amoretti, C. Muryn, **F. Tuna**, G. Timco, E. J. L. McInnes, R. E. P. Winpenny, W. Wernsforfer, M. Affronte, *Phys. Rev. Lett.* **2010***, 104***,** 37203/1 - 37203/4.

2. G. A. Timco, S. Carreta, F. Troiani, F. Tuna, E. J. L. McInnes, M. Affronte, R. E. P. Winpenny, *et al.*, *Nature Nanotechnology* **2009**, *4*, 173.

Membrane protein incorporation into novel polymer-lipid particles and their physical-chemical characterization

Marcella Orwick[*], Andrea Graziadei[*], Lubica Aslimovska[*], Daniele Selmi[**], Anthony Watts[*]

[*]Biochemistry Department, University of Oxford, South Parks Road Oxford OX1 3QU
[**]Physics Department, University of Oxford, Parks Road, Oxford OX1 3PU

Incorporation of membrane proteins into discreet lipid particles provides an excellent environment for detailed structural analysis as micellar solutions are poor mimics of a lipid bilayer. Here we report on the physical chemical characterization of a novel styrene-maleic acid polymer-lipid complex that is able to effectively form lipid particles, termed lipodisqs (1,2,3), and the incorporation of the well-characterized protein Bacteriorhodopsin, a 7TM 26 kDa transmembrane protein that exists as a trimer in the purple membrane (PM) of *Halobacterium salinarium* and functions as a light-driven proton pump (4,5). Tempo titration and lipid ordering experiments involving spin-labelled styrene maleic acid have confirmed the lipodisq lipid ordering and phase transition, and TEM and DLS were both employed to determine the size. Bacteriorhodopsin was functionally reconstituted into lipodisqs in a monomeric form as confirmed by TEM, CD, and UV-Visible spectroscopy. Preliminary cw and pulsed EPR data are presented on the protein dynamics and structure.

1. Tonge, S. R. and Tighe, B. J. *Adv. Drug Delivery Rev*. 2001, 53, 109– 22.
2. Tonge, S. R. Patent GB2426703, WO2006/129127 2006.
3. Knowles TJ*, et al.* (2009*J Am Chem Soc* 131(22):7484-7488.
4. Oesterhelt D & Stoeckenius W (1971). *Nat New Biol* 233(39):149-152.
5. Oesterhelt D & Stoeckenius W (1973) *Proc Natl Acad Sci U S A* 70(10):2853-2857.

Comparative ESR analysis of impurity doped ammonium selenate single crystals

Ashutosh Kumar Shukla, Ram Kripal*

Department of Physics, Ewing Christian College, Allahabad, India, 211003
** Department of Physics, University of Allahabad, Allahabad,India, 211002*

A comparative ESR study of manganese and vanadyl doped ammonium selenate single crystals is presented. ESR experiments have been performed at X-band and room temperature. Different spin-Hamiltonian parameters have been determined. Site symmetry and location of impurity ions in the lattice has been ascertained. Powder spectra have also been recorded and analyzed.

Title index

Destination State Screening of Active Spaces in ESR Simulations. P49

Detection and characterization of superoxide radicals by using spin trapping method with DEPMPO. P37

Development of an electron spin-based biosensor. P50

Distance Measurements by DEER in Gd^{3+} – Nitroxide Radical Spin Pairs. P10

Distance Measurements on Lanthanide Ion – Nitroxide Radical Spin Pairs in the Nanometre Range by Relaxation Enhancement. T10

DNA Condensation observed by Multi-Spin-Probe Techniques. P21

Dynamic effects on the EPR spectra of propagating methacrylic radicals. P13

Dynamics and topology of spin-labeled lipids and peptide antibiotics in model phospholipids membranes probed by stimulated ESE. T15

ELDOR study of the full-length Agp1 phytochrome from *Agrobacterium tumefaciens*. P36

Electron Spin Resonance Studies on Copper Triangles. P23

Electron transfer in proteins of the photolyase/cryptochrome family: Light-induced radical-pair formation is more complex than initially anticipated. P45

Electronic structure of large polymetallic molecules: a multi-frequency EPR approach. T41

ENDOR measurements on *Arabidopsis thaliana* Cryptochrome-1. P19

EPR and spin relaxation of charge carriers in π conjugated polymer fullerene blend thin films. P15

EPR investigations on mutants of the membrane-bound [Ni-Fe]-hydrogenase from *Ralstonia eutropha.* T43

EPR Studies of Titania Photocatalysts for Organic Synthesis. P12

EPR study in magnetoresitive $GdBaCo_2O_{5+\delta}$. P5

EPR study of Mn centres in $PbTiO_3$ single crystals. P14

EPR, MS, XRD and TEM study of $Mn_xZn_{1-x}Fe_2O_4$ nanoparticles. P7

EPR/Alanine Dosimetry: Final Chapter? T40

ESR-spectroscopy in ionic liquids: Investigations on the dynamics and kinetics of organic radicals. T48

Exploring the Binding Site of Ubiquinone in Complex I by EPR/DEER. P4

Exploring the energy landscape and dynamic modes of a protein with site directed spin labelling. T25

Generation and detection of an entangled state for synthetic electron spin-qubits. P31

Ghost distances in double electron-electron resonance of multiple-spin systems. P3

Graphical user interface for the specification and visualization of interactions in large spin systems. P51

Participating Author Index

Nick		Kassouf	P42, T38
Daniel		Kattnig	P44, T48
Chris	W. M.	Kay	P2, P30, P34, P35, P48, P50, T32, T38
David	J.	Keeble	P14, P15
Johann	P.	Klare	P2, P35, P39, T26
Zoltán		Klencsár	P7
Daniel		Klose	P2, P35
Ram		Kripal	P55
Ivan		Krstic	T9
Micha	B. A.	Kunze	P30, P34, P48, P50, T32
Ilya		Kuprov	P49, T34, P51
Dennis		Kurzbach	P21, T7
Petra		Lüders	P10, T10
Fraser		MacMillan	P22, P46, T13
Hassane	S.	Mchaourab	P26, T12
Eric		McInnes	P29, T20, T36, P53
Elisabetta		Mileo	T6
Deborah	G.	Mitchell	T18
Mohamed	A.	Morsy	T40
Damien	M.	Murphy	P11, T42
Nóra	V.	Nagy	P25
Shigeaki		Nakazawa	P31
Thomas		Newby	P52
Mark	E.	Newton	T19
David	G.	Norman	P40, T17, T27
Vasily	S.	Oganesyan	T33
Cesare		Oliva	P5
Marcella		Orwick	P54
Dipali		Patel	P34
Katharina	F.	Pirker	P30, P34, P48, P50, T32
Christos		Pliotas	P20, P41
Thomas	F.	Prisner	P27, T9, T24
Susanne		Pudollek	P1
Kenneth		Rasmussen	P17
Gunnar	W.	Reginsson	T17, T30
Maxie	M.	Roessler	T11
Kacper	B.	Rogala	P48
Vishal	A.	Sanchania	P50
Olav		Schiemann	P20, P38, P41, T30, T17
Daniel		Sells	P29
Maryam	H.	Shabestari	T47
Ashutosh	K.	Shukla	P55
Snorri	Th.	Sigurdsson	T5, T30
Stephanie		Simmons	T35
Graham	M	Smith	P20, P31, T17, T30
Lorenzo		Sorace	T21
Eike	T.	Spielberg	P23
Stephen		Sproules	T22

Heinz-Jürgen		Steinhoff	P2, P35, P39, P47, T26
Stefan		Stoll	T46
Klaus		Stolze	T3
Dimitri		Svistunenko	T14
Christian		Teutloff	P1, T43
Christiane	R.	Timmel	P43, T44, P28
Yuri	D.	Tsvetkov	T28
Floriana		Tuna	T36, P53
Tona		von Hagens	P3
Richard		Ward	P20, P40, P41, T17, T27
Stefan		Weber	P4, P36, P45, T45
Christopher	J.	Wedge	P23, T36
Ringo		Wenzel	P19
Helen	E.	Williams	T39