epr newsletter

2007
volume 17 number 2-3

The Publication of the International
EPR (ESR) Society

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Please feel free to contact us with items (news, notices, technical notes, and comments) or ideas for the *EPR newsletter*.

The *EPR newsletter* is published quarterly by the International EPR (ESR) Society and is available in electronic and printed form to all members of the Society. The deadlines for submission of news for upcoming issues: Spring March, 15; Summer June, 15; Fall September, 15; Winter December, 15.

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The cover picture is dedicated to the research of George Feher, who shared the 2007 Wolf Prize in Chemistry with Ada Yonath "for ingenious structural discoveries of the ribosomal machinery and the light-driven primary processes in photosynthesis".

Taken by Daniella Goldfarb

epr newsletter

The Publication of the International EPR (ESR) Society

volume 17 number 2-3 2007

ETH
Eidgenössische Technische Hochschule Zürich
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Reader's Corner

Dear Laila,

A new website has been set up for the European Federation of EPR Groups (EFEPR) to offer what we hope will be a platform for the EPR community for information and communication, and everyone is invited for a visit at www.physics.ua.ac.be/EFEPR. The items from the previous website are incorporated, but there are also novelties, including a level of interactivity. As indicated on the website, announcements of 'Jobs' and 'Conference/Meetings' can be submitted, and furthermore we welcome contributions of 'News' items and others information of interest to the EPR world, that can be sent for addition to the site.

Etienne Goovaerts
President of the EFEPR

Editorial

Dear colleagues,

I can imagine that it took you some time before you could stop looking at the cover picture and turn the page to read the list of contents of this *newsletter*. The warm brown color of the olive tree trunk, the depth of its folds and the variety of curvatures are bewitching. How old is this olive tree patriarch? What story is behind it? It might date back to Moses times. At the same time, the fresh green of a young shoot predicts its glorious future. The photo was taken by Daniella Goldfarb to illustrate George Feher's research that led to the Wolf Prize of Israel (for details see p. 6). In scripture Israel is often referred to as the olive tree. In Hosea 14 you find "His splendor will be like an olive tree". Feher's invention of ENDOR and impressive work in research of photosynthesis are his ingenious contributions to science. We are happy that once again we can pay tribute to one of our greats, George Feher (see also an ENDOR-related issue of the *EPR newsletter* 16/2-3). George, our congratulations and best wishes!

It is the holiday season and all of you worked very hard in 2007. You deserve to get presents. First, you must have received copies of the *newsletter* 16/4 and 17/1. They were printed with some delay because we had to be sure that a proper number of copies be printed and to this end, the number of paying members was checked. We also prepared a special present for you, a double issue of the *newsletter*. Just look at these colorful boxes under the beautifully decorated evergreen Christmas tree! You start opening them one by one anticipating the joy of discovering new exciting things and here you are! You find a detailed report about the IES Annual meeting 2007. For the first time it took place in Europe, in Oxford, at the 40th Annual ESR Conference. The charm of Oxford, with its magnificent architecture and green lawns with lots of daffodils added to the success of this meeting. What are you doing? You are looking for the box with the 'Another Passion' column and you do not find it? You are disappointed, aren't you?! Me too! I am sorry but this time I failed to get the relevant material.

Come on, my dear, you cannot have everything. To compensate, in this *newsletter* we publish the remaining two parts of Bill Mims' reminiscences in the '*EPR newsletter* Anecdotes' column. You can join us congratulating our outstanding colleagues on their anniversaries. The relevant articles give details of their background and impressive achievements. I had the pleasure of meeting all of them in person. I could underline what they all have in common: deep and very interesting personalities. The biggest box contains a comprehensive and wonderful 'Pro & Contra' review on dipolar methods from Peter Borbat and Jack Freed. To keep you in a proper mode of excitement I won't tell you about the contents of other boxes you see under the Christmas tree. Start reading the *newsletter* and you will find out everything!

As a final touch, I would like to update you about an initiative of Wayne Hubbell. He plans to cover the IES membership dues for his young collaborators. In my opinion, it is vital to involve the younger EPR generation in the IES activities and this first step could stimulate their interest in the IES. Later on, if they think it is worth it, they could cover their membership dues themselves. I think it would be helpful if all our IES members could afford to follow Wayne's initiative.

Happy New Year to you, our dear readers! I wish you all the best! Please feel free to contribute your news to the *EPR newsletter*. Your inputs are always welcome!

Laila Mosina

IES Annual Meeting 2007

Held at the 40th International ESR Conference of the Royal Society of Chemistry ESR Group, Oxford, UK on March 26th, 2007. The meeting was opened and chaired by the President of the Society, Professor Wolfgang Lubitz and opened at 17-00.

1. Attendance and Apologies

Attendance (42): Angelo Alberti, Elena Bagryanskaya, Riccardo Basosi, Stephen Bingham, Marina Brustolon, Yiannis Deligiannakis, Gareth Eaton, Sandra Eaton, Shirley Fairhurst, Ruslan Garipov, Stephane Grimaldi, Daniella Goldfarb, Etienne Goovaerts, Edgar Groenen, Wilfred Hagen, Graeme Hanson, Ping Huang, David Keeble, Wolfgang Lubitz, Fraser MacMillan, Kiminora Maeda, Eric McInnes, Keith McLauchlan, Jacek Michalik, Klaus Möbius, Gavin Morley, Mohamed Morsy, Laila Mosina, John Pilbrow, Thomas Prisner, Boris Rakvin, Edward Reijerse, Yiannis Sanakis, Heinz-Jürgen Steinhoff, Les Sutcliffe, Dima Svistunenko, Christian Teutloff, Yuri Tsvetkov, Chris Wedge, Hogny Weihe, Lev Wiener, Dmirty Zverev.

Apologies: Chris Felix, Balyanaraman Kalyanaraman and Shozo Tero Kubota.

2. 2006 Minutes

The minutes of the General Meeting held on the 26th July 2006 were presented and accepted as a true record of the previous meeting.

3. President's Report – presented by Wolfgang Lubitz

Dear Colleagues,

On behalf of the IES Executive Committee, I wish to welcome all participants to the 2007 General Meeting of the IES and the 40th International EPR Conference of the ESR Group of the Royal Society of Chemistry. I would like to express my gratitude to the ESR Group for allowing our General Meeting to take place during this Conference. This is the first time we have held our General Meeting outside the USA and reflects the worldwide membership of the Society.

We are here to tell you about our work over the past year but also to hear your views.

- IES Awards 2007:
  - Silver Medal (Physics/Materials Science): awaiting decision from committee
  - Young Investigator Medal: Dr Leonid Kulik (Novosibirsk, Russia)
  - Fellow of the Society: Prof. Les Sutcliffe (Norwich, UK)
- During my first year of presidency of the IES, I represented the Society at the following meetings:
  - ANZMAG meeting, February 2006, Australia
  - RSC EPR group meeting, April 2006, Edinburgh, UK
  - 29th EPR Symposium, July 2006, Breckenridge, USA
  - International Conference of MR in Biological Systems, September 2006, Göttingen, Germany
  - 6th European Federation of EPR Groups Meeting, September 2006, Madrid, Spain
  - International Symposium on Modern Problems in Chemical Physics, Oct/Nov 2006, Kazan, Russia
  - RSC ESR group meeting in March 2007, Oxford, UK

During my visit to Australia, I presented the award of Fellowship of the IES (2006) to John Pilbrow (Monash University). Here in Oxford the Fellowship of the IES (2007) was awarded to Les Sutcliffe (Norwich, UK). At the 2006 EPR Symposium in Breckenridge USA, the Silver Medal in Biology/Medicine was awarded to Periannan Kuppusamy (Columbus, USA). During the 2006 EFEPR meeting in Madrid I presented the Silver Medal for Physics/Instrumentation (2005) to Jos Disselhorst (Leiden, Netherlands) and the Young Investigator Award (2005) to Eric McInnes (Manchester, UK).

The Silver Medal in Biology/Medicine (2006) will be given to Jay Zweier (Columbus, OH) at the "EPR 2007" meeting in Chicago. I plan to award the Young Investigator Medal 2007 to Leonid Kulik (Novosibirsk, Russia) in July at the VII Voevodsky Conference in Chernogolovka, Russia. The decision on the award of the Silver Medal 2007 is still pending.*

Out of the many awards and honours given to members of the IES by other so- ▶

* The IES Silver Medal 2007 was awarded to Prof. Thomas Prisner (Frankfurt), see p. 7 for details.

IES Awards 2008

Call for Nominations

Nominations are invited for the following Awards: Gold Medal, Silver Medal for Instrumentation and Fellowship of the Society (see extract from by-laws below or visit ieprs.org for full constitution and by-laws).

All nominations must be accompanied by a 100–150 word citation in support of the nomination. No nomination can be considered without a citation. Additional supporting material may be included.

Nominations are to be sent in confidence to the President by e-mail in doc, rtf or pdf format to: lubitz@mpi_muelheim.mpg.de. Please put the words: *Confidential IES Award Nomination* in the title; or by mail to: Prof. Dr. Wolfgang Lubitz, IES President, Max-Planck-Institut für Bioanorganische Chemie, Stiftstr. 34-36, D-45470 Mülheim an der Ruhr, Germany.

The closing date for nominations for Awards in 2008 is 31st December 2007.

By-laws

The Gold Medal shall be awarded for distinguished contributions to EPR (ESR) Spectroscopy.

The Silver Medal shall be awarded for significant contributions to EPR (ESR) Spectroscopy in the subject area of the Award.

The Fellowship of the Society may be conferred on individuals who have made influential and distinguished contributions to the practice of EPR (ESR) Spectroscopy and its welfare over a long period.

cieties and institutions I want to mention just three:

Early in 2006 Brian Hoffman became member of the National Academy of Sciences of the USA. In November 2006 Klaus Möbius was awarded the Bundesverdienstkreuz 1. Klasse of the Federal Republic of Germany in Berlin. This year (May 2007) George Feher will be awarded the prestigious Wolf Prize in Jerusalem, Israel.

Our sincere congratulations to all three Scientists!

- Conferences 2007:

June 17–22 2007: Gordon Research Conference – Magnetic Resonance University of New England, Biddeford ME, USA www.grc.org/programs.aspx?year=2007&program=magres

July 22–26 2007: ESR Symposium at the 49th Annual Rocky Mountain Conference, Breckenridge, CO, USA www.rockychem.com/epr/index.htm

August 12–17, 2007: 29th International Symposium on Free Radicals Big Sky, Montana, USA www.freeradicalssymposium.org

September 1-6, 2007: ECSBM 2007, the European Conference on the Spectroscopy of Biological Molecules Bobigny (Paris), France www.ecsbm.eu

September 2–6, 2007: 5. International Conference on Peroxynitrite and Reactive Nitrogen Species Montevideo, Uruguay www.cfrbr.fmed.edu.uy

September 24–29, 2007: Modern Development of Magnetic Resonance "Zavoisky-100" Kazan, Russia www.kfti.knc.ru/zavoisky100

September 26–29, 2007 : 29th meeting of FGMR, the German Magnetic Resonance Spectroscopy Group of GDCh Göttingen, Germany fgmr.chemie.uni-hamburg.de/events

October 3–6, 2007: VIIth International Workshop on EPR (ESR) in Biology and Medicine, Krakow, Poland eprworkshopkrakow2007.xt.pl

October 14–19, 2007: ISMAR 2007 Kenting, Taiwan www.ismar2007.sinica.edu.tw

November 6–9, 2007: 46th Annual (International) Meeting of the Society of Electron Spin Science and Technology (SEST 2007), Shizuoka, Japan

Finally I want express my gratitude to the medal committees (this year Silver Medal Physics/Materials Science) for their excellent and sometimes very difficult work. In the name of the whole IES I thank also the secretary, Shirley Fairhurst, for her great support of the president and Laila Mosina for her truly outstanding contribution to the EPR Newsletter and Chris Felix as treasurer of the IES. Each time the president cannot take part in an event important for the IES, one of the vice presidents or the past president takes over. Their work – also as part of the IES Executive - is gratefully appreciated!

4. Secretary's Report – presented by Shirley Fairhurst

- **IES Awards 2008. Call for Nominations**

Nominations are invited for the following awards: Gold Medal, Silver Medal (Instrumentation), and Fellow of the Society (visit ieprs.org for full constitution and by-laws). All nominations must be accompanied by a 100–150 word citation in support of the nomination. No nomination can be considered without a citation. Additional supporting material may be included.

Nominations are to be sent in confidence to the President.

Closing date: 31st December 2007.

- **IES Executive Elections 2008**

In October 2008 the current IES Executive's three year term of office will end. Nominations are sought for the following posts: President, Vice President Americas, Vice President Asia Pacific, Vice President Europe, Secretary and Treasurer.

The official call for Nominations will appear in Newsletter 17/2, with a closing date of 31st December 2007.

5. Treasurer's Report – presented by Wolfgang Lubitz on behalf of Chris Felix

Membership forms are included in the handouts or join via the web site.

A series of screen shots was shown (see Newsletter article on using the IES web site members pages) on how to login to the Society's web site (www.ieprs.org) which ▶

The EPR community has available to it a list server. The address is epr-list@xenon.che.ilstu.edu.
To subscribe to the list, send the words SUBSCRIBE epr-list to majordomo@xenon.che.ilstu.edu.
That sends a message to Reef Morse who will then manually place you on the list. This honors only legitimate requests to join the list. Reef also moderates the list which keeps it spam-free.

would allow members to check whether their membership was current, pay for past and future years and also change their personal details.

2006 Financial Report ($) (unaudited)

Balance January 1, 2006	1918.64
Income:	
Total Income	10613.55
Expenses:	
Bank & credit card fees	609.44
Web design & fees	436.25
Newsletter	8307.00
Awards	130.00
State of Illinois	5.00
Total Expences	9487.69
Balance December, 31, 2006	3044.50

Membership

If you are interested in joining the IES	
Membership fees ($) are:	
Full	30
Emeritus/retired	10
Postdoctoral (3 years max)	10
Student	5

6. Newsletter Editor's Report – presented by Laila Mosina

Since the previous IES Annual Meeting in 2006, the EPR newsletter 16/2-3, a double issue dedicated to the 50th anniversary of the discovery of ENDOR by George Feher, was published and the pdf version of 16/4 was prepared.

Laila Mosina presented the preview of the EPR newsletter 16/4. The cover pictures of 16/2-3 and 16/4 illustrate research of recipients of the IES Silver Medal for Instrumentation 2005 (Jos Disselhorst) and Zavoisky Award 2005 (Hal Swartz), respectively.

On behalf of the Editorial Board, she thanked most heartily all contributors to the EPR newsletter with special thanks going to the CEOs of the IES and editors of the columns in the EPR newsletter: Shirley Fairhurst, John Pilbrow, Candice Klug and Thomas Prisner, and also to Stefan Stoll, our web-master and Sergei Akhmin, our Technical Editor. Laila is most appreciative of the support from Shirley Fairhurst, which provided her with the opportunity to participate in the Oxford conference.

Laila Mosina gratefully acknowledged collaboration with Associate Editors Candice Klug, Hitoshi Ohta and Thomas Prisner.

7. Thanks

The IES thanks the following Corporate Sponsors for their contributions in 2005-2006:

Bruker BioSpin Corp.
JEOL USA, Inc.
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Research Specialties
Resonance Instruments Inc.
Wilmad-LabGlass
Update Instrument, Inc.
Molecular Specialties, Inc.
Scientific Software Service
L&M EPR Supplies, Inc.
Norell, Inc.

8. Any Other Business

Membership: It was noted that many members are in arrears with their membership. It was announced that members who have not paid for three years or more will become 'inactive members' and will not receive the printed version of the EPR Newsletter or the password for the downloadable copies. Members who are more that one year in arrears will not receive the printed version of the EPR Newsletter from issue 17.

Awards: There was some discussion led by Wolfgang Lubitz regarding the possibility of future IES Medalist receiving a monetary prize as well as their medal and certificate. This will depend on the health of the Finances.

Executive Continuity: Concern was expressed that in 2008 all the IES Executive will change. It was proposed that the current Executive consider this situation and in the first instance that they each try to find members willing to take over their roles (bearing in mind the need for geographical diversity etc.).

Date of next meeting: During APES July 13–18 2008 Cairns, Australia.

Are you interested to become a member of the International EPR (ESR) Society? Please find the registration/ information form for new/ continuing members of the IES and non-credit-card payment instructions for individual members on this Web site: www.ieprs.org

IES Executive Elections Office Bearers for October 2008 – September 2011

Call for Nominations

In October 2008 the current IES Executive's three year term of office will end. Nominations are sought for the following posts:

– President
– Vice President Americas
– Vice President Asia Pacific
– Vice President Europe
– Secretary
– Treasurer

Our Constitution Article VIII. Elections, reads: "Nominations for all positions of Office Bearers shall be made by the Executive that shall have regard to geographical and international distribution of nominees. Nominations may also be made by at least ten paid-up members of the Society, in writing to the Secretary, and received by a date specified with appropriate notice in the official Bulletin or Newsletter of the Society. Where there are one or more nominations for any position, the Elections Committee shall conduct the election according to the provisions following in clauses 2 and 3."

There are thus two ways a person may be nominated for Office in the Society. The current Executive is required to make nominations for all positions. Nominations can also be made by 'at least ten paid-up members of the Society' for all elected positions: President, Vice-President Americas, Vice-President Asia/Pacific, Vice-President Europe, Secretary and Treasurer.

Nominations in writing should reach the Secretary, Dr Shirley A. Fairhurst, John Innes Centre, Norwich Research Park, Colney, Norwich NR4 7UH, UK (shirley.fairhurst@bbsrc.ac.uk) by post, e-mail or fax (+44 (0)1603 450018) before 31st December 2007.

George Feher Receives the Wolf Prize at the Israeli Knesset

George is receiving a present from the Weizmann Institute and is addressing the people at the dinner.

On May 13th George Feher was awarded the 2007 Wolf prize in Chemistry, shared with Ada Yonat from the Weizmann Institute. The Prize Awarding Ceremony took place at the Knesset (Israeli parliament) Building in Jerusalem. The awards were presented to the recipients by the President of the State of Israel, in the presence of the Chairman of the Knesset and the Minister of Education, Culture and Sport.

The prize citation: "His ingenious contributions to science are centered on two recurrent themes, which address the development of novel and revolutionary spectroscopic tools, on the one hand, and their applications, in particular, to problems in biochemistry and biophysics, on the other.

George with Ada Yonat and Haim Garti, the Vice-President of the Weizmann Institute

George Feher's invention of electron-nuclear double resonance (ENDOR), as an example of only one of the novel methods, opened a field of applications, the enormous breadth of which only became apparent in the course of time. This method allows one to obtain detailed information on structure both for polycrystalline and amorphous materials. Due to these features ENDOR has become a great asset in the study of biological systems with paramagnetic centers.

Although the existence of a 'reaction center' in photosynthesis was postulated as early as 1952, its true nature became apparent only through the work of a later generation of scientists from all over the world. Feher was the first to identify the amino acid sequence of a membrane protein, and built the essential steps of developments that led to the present detailed understanding of the reaction center, including its structure. Feher's impressive work in research on photosynthesis rests on his extraordinarily vivid imagination and on the sustained discipline with which he forced himself to master the underlying biochemistry in a brilliant and systematic manner. These qualities allowed him to view the complex problems related to the primary steps of photosynthetic energy conversion in their entirety, while many specialists tended to concentrate only on individual pieces of the puzzle.

Since insight into the structure and the charge separation mechanism of the reaction center has provided the principles of optimized light energy conversion in biology, Feher's work is seminal for the construction of synthetic and semi-synthetic molecular energy converters, which may have profound implications in an energy-demanding world."

Since 1978, five or six prizes have been awarded annually in the Sciences. Prize fields comprise: Agriculture, Chemistry, Mathematics, Medicine and Physics. The prize in each field consists of a certificate and a monetary award of $100,000. In the event of two or three recipients sharing the prize, the honorarium is divided equally. Many of the Wolf prizewinners were awarded later the Nobel prize!

Other recipients of the Wolf prize from the field of magnetic resonance are: Richard Ernst and Alex Pines – Chemistry 1991. Herbert S. Gutowsky, Harden M. McConnell and John S. Waugh – Chemistry 1983/4. Erwin Hahn – Physics 1983/4.

Elsa Feher, Klaus Möbius and George Feher

One Laureate in each field delivers a short acceptance speech and George spoke on behalf of the Chemistry Laureates. George delivered a very special speech; its scientific part was delivered in English, but personal notes were given in Hebrew. He described his days in Israel in the early 1940's and his attempts to enroll into the Engineering school of the Israeli Technical Institute (Technion) in Haifa. George was not accepted because he did not pass the Bible entrance exam… and therefore he had to go to school in the USA; Israel lost a wonderful and brilliant scientist. Nonetheless, George has always kept close contacts with Israel and its scientific community.

The prize ceremony has brought to Israel also close friends of George among them Klaus Möbius, Wolfgang Lubitz, Giovanni Giacometti and Maibi Michel-Beyerle. There were two mini symposiums in the honor of the Wolf Prize Laureates, George Feher and Ada Yonat, one at the Weizmann Institute and the other at the Technion. I attended the one at the Weizmann Istitute where Klaus gave a wonderful talk "Multi-frequency EPR on Photosynthetic Reaction Centers: Picking Flowers at Crossroads to George Feher". Due to some technical difficulties Ada Yonat changed Klaus's slides… did any of you have a Wolf Prize Laureate change his/her slides?

Unfortunately, I could not take pictures at the ceremony in the Knesset; due to security we could not bring cameras. But I did take a couple of pictures at a dinner given by the Vice president of the Weizmann Institute in honor of the Prize recipients.

Finally, a personal note – this year's Wolf prize award was a very special event for me because I know both Laureates personally; George Feher is the inventor of my major research technique and Ada Yonat is a friend and a member of my Faculty (and is a woman!).

Daniella Goldfarb

The Bruker Prize 2007 to Daniella Goldfarb

From left to right: Dieter Schmalbein (Bruker BioSpin), Daniella Goldfarb (2007 Bruker Prize), Shirley Fairhurst (ESR Group Chair) and Peter Höfer (Bruker BioSpin).

For details, see this newsletter, p. 34.

2007 IES Silver Medal for Physics/Materials Science to Thomas F. Prisner

Thomas Prisner has made major contributions to the development of advanced multi-frequency pulsed EPR spectroscopy and its extension to double resonance techniques such as ENDOR and PELDOR.

In particular, his deep understanding of pulsed high-field EPR, ENDOR and PELDOR spectroscopy have enabled new applications to highly interesting systems from biochemistry and molecular biology. Prominent examples of his accomplishments in biophysics and material science are found in the areas of the kinetics and dynamics of electron-transfer reactions in proteins, conformational dynamics of macromolecules, reaction dynamics and structures of catalytic metal centers in enzymes, protein-ligand and protein-protein interactions, and structure determination of paramagnetic centers in proteins.

His research has stimulated many groups in Europe, the US and Japan in their own pulsed high-field EPR work. He is currently one of the driving forces of the DNP (Dynamic Nuclear Polarization) project (together with the University of St. Andrews and the Bruker Biospin Company) for the development of ultra-sensitive solid-state NMR spectroscopy.

In recognition of his many contributions to advanced multifrequency pulsed EPR Professor Thomas Prisner richly deserves the Silver Medal of the International EPR (ESR) Society.

Thomas Prisner (left) and Wolfgang Lubitz (right).
The XIth Chianti Workshop on Magnetic Resonance in Vallombrosa (Florence), Italy, June 3–8, 2007.

2007 IES Young Investigator Award to Leonid V. Kulik

Leonid Kulik (Institute of Chemical Kinetics and Combustion, Novosibirsk, Russia) is awarded the 2007 Young Investigator Medal for his studies in pulse EPR spectroscopy, including the development of novel pulse EPR methods and their application to chemical and biological systems.

At the Institute of Chemical Kinetics and Combustion he studied ESEEM effects in stable nitroxide radicals and found a new type of ESEEM, which is caused by spontaneous alternation of the Larmor frequency of the electron spins. In biradicals where spin-lattice relaxation alternates the dipolar interaction between two spins, the resulting ESEEM effect was called Relaxation-Induced Dipolar Modulation Enhancement (RIDME). Leonid also participated in the development of field-step ELDOR as applied to study dipolar interaction in biradicals.

As guest scientist at the Huygens Laboratory of Leiden University, The Netherlands, he applied out-of-phase ESE spectroscopy to investigate spin-correlated radical pairs and triplet-radical pairs, which appear after the light-induced electron transfer in the bacterial photosynthetic reaction centers. He detected new signals: out-of-phase FID in selective hole-burning experiments and out-of phase stimulated echo.

Leonid Kulik. The VII Voevodsky Conference "Physics and Chemistry of Elementary Chemical Processes", Chernogolovka, Russian Federation, June 24–29, 2007.

At the Max Planck Institute for Bioinorganic Chemistry, Mülheim an der Ruhr, Germany, Leonid Kulik worked on pulse EPR and Mn-ENDOR of the Oxygen Evolving Complex (OEC) of plant Photosystem II. He successfully applied pulse Q-band spectroscopy to study the OEC in two paramagnetic states. These results yielded the overall composition of the oxidation states of the Mn ions in the OEC and together with recent EXAFS data resulted in a refinement of the OEC model and the proposal of a detailed mechanism of photosynthetic water splitting.

IES Young Investigator Award Revisited

This column features former recipients of the IES Young Investigator Award.

EPR on Macromolecules

After receiving the 1996 Young Investigator Award for my PhD work on hyperfine spectroscopy, I was of course tempted to stay in this field and build a career on this early success. However, I followed good advice by my PhD supervisor Arthur Schweiger to 'go somewhere where you can learn something new'. After briefly flirting with solid-state NMR I took an offer by Hans W. Spiess to join his department of polymer spectroscopy at the Max Planck Institute for Polymer Research (MPI-P) in Mainz in 1998. I was quite sure that polymer physics was something new to me and that I did not know much about EPR of nitroxides either. Other attractions of this non-permanent project leader position were the efficient administration of Max Planck institutes, a rather generous budget, and limited teaching duties. Nowadays I like teaching a lot, but in my early thirties I considered it as a distraction from research. Nevertheless, my first lecture script ("Introduction to EPR" in German) was downloaded in Zurich and induced Arthur's invitation to join him in writing a book on pulsed EPR spectroscopy (1998–2001). In due course I found out that I could still learn a lot of new things from Arthur, too.

Before I came to Mainz the EPR group at MPI-P had already started with distance measurements on synthetic macromolecules. My first encounters with polymer physics convinced me that this whole branch of science is about distributions of measurable quantities. Therefore we had to learn how to extract distance distributions from our EPR data. Fitting one or two Gaussian peaks to the data was our first approach but proved unsatisfying for some of the interesting problems. Through conferences in 2001 we first proposed a model-free approach based on a specifically tailored fast integral transformation. Except for fast data pre-processing this algorithm is meanwhile superseded by Tikhonov regularization. Nevertheless studying the properties of the integral transformation helped us to understand the advantages and disadvantages of model-free approaches. Model-free extraction of distance distributions became a rather active field of research with contributions from the groups of Bowman, Freed, and Tsvetkov as well as ours (2001–2005).

Analysis in terms of distance distributions requires low-noise data. Thus we worked a lot on improvement of measurement protocols. These improvements finally made it possible to perform the first double electron electron

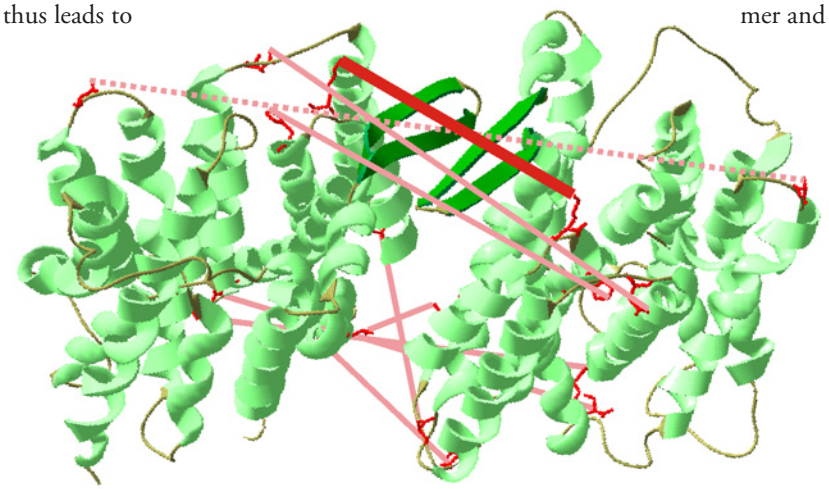
The Jeol Young Investigator Prize to Sharon Ruthstein

From left to right: JEOL Student Talk Prize: Chris Rodgers (University of Oxford), Peter Meadows (JEOL), Sharon Ruthstein (Weizmann Institute) and Olivier Rival (University of Oxford).

For details, see this newsletter, p. 34.

resonance (DEER) distance measurements on an integral membrane protein (2004). Again MPIP-P proved to be a good place to work at. Although its mission is polymer research, I was encouraged to venture further into biostructural research. Indeed we soon found that the techniques developed for synthetic polymers were also suitable to study conformational distributions in biomacromolecules. I decided to share time between materials science and biostructural work. In work on concatenated macrocycles (2003) and semiflexible polymers (2006) model-free extraction of distance distributions provided the basis for creating a structural model. However, this type of data analysis involves solution of an ill-posed problem. Direct fitting of the structural model to the primary data thus leads to more reliable results at a later stage. This two-step approach was again originally applied to synthetic polymers but is now our new standard approach to membrane proteins.

In work on the persistence length of semiflexible polymers we found that we had to explicitly account for the spin label and its conformational distribution. Several groups, among them Fajer's, Hubbell's, Perozo's, and Steinhoff's came to the same conclusion in work on biomacromolecules. In our own current work on membrane proteins we model this conformational distribution by a rotamer library. This approach provided a highly resolved structure of the Na^+/H^+ antiporter NhaA of *E. coli* (see figure). The docking problem could be solved using the x-ray structure of the monomer and nine site-to-site distance measurements by DEER (2007). Other concepts from polymer physics are now in the pipeline for future use in our biostructural work.



High-resolution structure of the Na^+/H^+ antiporter NhaA of *E. coli* derived from DEER measurements of nine site-to-site distance distributions (red solid and dashed lines) and modeling of the conformational distribution of the spin labels by a rotamer library [D. Hilger, Y. Polyhach, E. Padan, H. Jung, G. Jeschke: Biophys. J., 93, 3675–3683 (2007)].

Meanwhile my position at MPI-P had become permanent (2004), but my venture into several fields eventually required more resources than were available. When looking for a new position the combined work on polymer and biostructural applications proved to be an asset. The chemistry department at small-but-beautiful University of Konstanz is pursuing both materials chemistry and chemical biology and was looking for a full professor in physical chemistry who could cooperate with both 'wings'. Although they did not have an EPR group before they offered me this position. I gladly took it, almost exactly ten years after receiving the Young Investigator Award.

In retrospect my scientific life developed quite nicely in the past ten years. On the way it did not always feel like this. If I had to give any advice to the next generation I would first pass on Arthur's one to venture into new fields. Building cooperations with scientists within and, in particular, outside the field of EPR can also be safely suggested. My last tip, however, may be the most valuable one: never write an article for the EPR Newsletter column on past Young Investigator Awardees. You might suddenly feel old.

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Anniversaries

75th Birthday of James S. Hyde

Dr. James S. Hyde is a Professor in the Department of Biophysics at the Medical College of Wisconsin and Director of the National Biomedical EPR Center, an NIBIB Biomedical Technology Resource (P41) Center. He also serves as Adjunct Professor of Biomedical Engineering at Marquette University. In this role, several Marquette engineering students have carried out thesis and dissertation research in his laboratory. In 1989, Dr. Hyde was awarded the degree of Doctor Honoris Causa by Jagiellonian University in Krakow, Poland, in recognition of his many years of active collaboration with biophysicists from that institution. This collaboration remains active. He currently serves as PI of a Fogerty International Research Collaboration Award (FIRCA) grant entitled "EPR Sample Cell Resonator Design and Construction" which provides funds for support of research directed by his colleague Professor Wojciech Froncisz at the Department of Biophysics, Jagiellonian University. Dr. Hyde was inducted as a Fellow of the American Physical Society in 1975 and as a Fellow of the International EPR/ESR Society in 2002. In 1993, he was awarded the Gold Medal from the International EPR/ESR Society, and in 1999, he was awarded the Gold Medal from the International Society for Magnetic Resonance in Medicine. In 1989, he received the Bruker Prize by the Royal Society of Chemistry, and in 1995, he received the International Zavoisky Award.

Dr. Hyde obtained his PhD in solid state physics from the Massachusetts Institute of Technology in 1959. Following completion of his doctorate, he worked at Varian Associates in Palo Alto, California, from 1959 to 1975. At Varian, he rose through the ranks to become senior scientist and manager of EPR research and development. During these years, he served as mentor to eight postdoctoral fellows, several of whom became very well-established investigators. By the time he left Varian, he had published upward of 60 papers.

In 1975, Dr. Hyde was appointed Professor of Radiology at the Medical College of Wisconsin, and his collaborations with the Department are noteworthy today. He became Chief of the Biophysics Section of the Department of Radiology in 1983 and helped establish the Biophysics Graduate Program in 1982, serving as Chairman of the program from 1990 until 1998. In 1992, he founded the Biophysics Research Institute and served as Director of the Institute until 1998, at which time he stepped down to pursue his research interests full time. The Biophysics Research Institute achieved full departmental status in 2003.

In addition to helping establish the Biophysics Graduate Program, Dr. Hyde served as a mentor for doctoral students in both the EPR and MRI fields. Since 1982, Dr. Hyde has served as PhD advisor for 17 individuals, four of whom are his current doctoral students. In addition, he maintains extensive collaborations with a number of clinical departments and has mentored fellows in Radiology, Gastroenterology, and Plastic Surgery.

Dr. Hyde's scientific interests are extensive. He has support from two EPR based R01 awards as well as the P41 grant, which help contribute to the development of EPR instrumentation and extend the ways in which existing EPR instrumentation can be used for new categories of biomedical problems. His papers on multiquantum EPR and pulse Saturation Recovery EPR are not only examples of interesting spin physics, but also hold great promise for research using site-directed spin-labeling. His papers on time-locked sub-sampling (TLSS) detection bring modern digital signal acquisition methods to EPR spectroscopy. It is believed that the techniques described in these papers will serve as the foundation for the next generation of EPR spectrometers. He has developed a digital detection spectrometer for use at Q-band. This instrument can be used for multiquantum EPR and ELDOR, standard EPR, and saturation recovery and pulse ELDOR EPR. He is currently engaged in extending these capabilities to W-band using frequency-translation technology. He also is an expert in several areas of MRI technology including surface coils, gradient coils, and shim coils, and is currently focusing on 9.4 T imaging of the rat brain, where he perceives that underlying technology is underdeveloped in comparison with human imaging.

Dr. Hyde has authored more than 350 papers and holds 33 U.S. patents. These publications cover his two main fields of expertise: instrumentation and methodological development in EPR and MRI. He has also served as a member of the Journal of Magnetic Resonance, Magnetic Resonance in Medicine (Associate Editor), Applied Magnetic Resonance, and Review of Scientific Instruments (1992 to 1994) editorial boards. He served as Chairman to both the Resource for Quantitative Functional MRI Advisory Committee at Johns Hopkins University Medical School (2001 to 2007) and the Pittsburgh NMR Center for Biomedical Research at Carnegie Mellon University (1997 to 2006). He previously served on the advisory boards of both the Biomedical Magnetic Resonance Research & Technology Center at the University of Illinois at Urbana-Champaign (1992 to 2000) and the EPR Center for the Study of Viable Biological Systems at Dartmouth Medical School (1997 to 2005). He currently serves on the advisory board of the Center for EPR Imaging for In Vivo Physiology at the University of Chicago Medical School (2003 to present). From 1990 to 1993, he was Trustee of the International Society for Magnetic Resonance in Medicine, Chairman of the Publications Committee in 1993, and Co-Chairman to the XIII International Conference on Magnetic Resonance in Biological Systems in Madison, Wisconsin.

Dr. Hyde received scientific recognition on the occasions of his 65th, 70th, and 75th birthdays and reports he is looking forward to the next one.

Chris Felix

Jim Hyde's Recollections

Starting with his first paper in 1960, Jim has selected important spin physics problems that continue to be investigated with improved spectrometers and improved EPR methodologies, some of which Jim has innovated in the intervening years. In recent years, Jim and his coworkers have added the application of modern finite element calculations of microwave field distributions to Jim's intuition to guide resonator designs. Years ago, Jim reassured researchers in EPR with the phrase 'there are spins everywhere'. We look forward to Jim's continued contributions to developing new tools and new methods of interrogating nature via electron spins. – *Dr. Gareth Eaton, University of Denver*

Without Jim Hyde's efforts over a period of more than four decades, EPR would not exist as a technology useful for the biological applications we have managed to demonstrate. From ELDOR to Saturation Recovery to the loop-gap resonator and beyond, his contributions have been truly monumental. Thankfully, current evidence suggests that the flow of original ideas will continue for some time. – *Dr. Wayne Hubbell, University of California, Los Angeles*

Jim Hyde has made many significant contributions to the EPR field such as to the development/expansion/promotion of EPR instrumentation for a wide range of biophysical applications where polar liquids are to be studied.

Probably, the most significant contributions to the field have been his ability to suggest, then construct, and to inspire others to make use of his new instrumentation where the need is not even apparent. When I was a postdoc with him from 1966 to 1967, he developed a bimodal cavity to carry out ELDOR measurements on polar liquids. At the time, only a couple of uninspired solid-state examples of ELDOR measurements existed in the physics journals. I inquired as to what experiment required such measurements. He couldn't answer the question at the time but challenged me to find one. I was studying the effect of ionizing radiation on organic crystals and soon found that the relaxation exhibited by the radicals was well suited for analysis by ELDOR measurements. It was possible to separate out the spectra of minor components underlying major components, to measure different types of motion that the radicals were undergoing at low temperature (77 K), as well as other applications. This led to many years of funded research during my academic career at The University of Alabama. Others in the lab at Varian, at the time, were also challenged. J. Chien and J. Freed (on vacation) found significant uses of the working ELDOR spectrometer by studying nitroxide radicals. This started a wide range of studies with nitroxide spin labels, which have resulted in the solutions of many biochemical structures. His ability to pick high impact areas of research is remarkable. – *Dr. Lowell Kispert, The University of Alabama*

Jim Hyde is an outstanding example for striving always for both aspects: high standards of science and international interactions.

I have a personal recollection to tell. Forty years ago (1967) I met Jim Hyde and Jack Freed at the Free Radical Conference in Novosibirsk (Akademgorodok), where ENDOR was among the strong topics. There I experienced both of them as the great stars of ENDOR-in-solution, their recent achievement together with Gus Maki. Jack Freed, unfortunately, got sick and spent most of the time in the hospital. Hence, Jim Hyde became the lonely star, always surrounded by a flock of admirers. It was the time of the Vietnam War, and walking over a road bridge one could see endless cargo trains going to Vietnam, loaded with tanks and cannons, hardly covered by canvas sheets. The American and Russian conference participants, together with their international colleagues, were leaning over the bridge railings watching the trains, exchanging their thoughts and worries about their brothers and sons serving as soldiers in Vietnam. And they understood each other far better than one could have hoped considering the official political statements from both sides of the Iron Curtain.

And then came the conference dinner in the middle of the Siberian taiga of Akademgorodok: again, Jim Hyde was the star of the evening. Vodka, and more vodka was flowing, helping to exchange ideas and to establish new East-West collaborations and friendships. Some of them are even lasting until today, for example Jim Hyde's EPR sustaining links to Novosibirsk and Krakow.

Jim's contributions to human interactions were – and still are – as successful as his contributions to spin interactions. – *Dr. Klaus Möbius, Free University Berlin*

Perhaps the most surprising (and also the most productive!) aspect of Jim's career has been his superb contributions to biomedical problems, with an aspect on medical. When we began to work together in 1964 on the development and administration of the National Biomedical ESR Center at the Medical College of Wisconsin, Jim's knowledge (and perhaps his interest) of medicine was rather unadvanced. In the subsequent years, however, his many contributions to the field have been greatly facilitated by his very sophisticated grasp of key medical issues and problems. These contributions have been impressively augmented by excellent insights into the perspectives and needs of academic physicians. While Jim's contributions to medicine have been mostly in the field of NMR, he also has made very positive contributions to the development of biomedical EPR directed towards medical problems, including some key contributions to my research in the development of clinical EPR, especially in the development of resonators. The broadest smiles that I have seen from Jim (and he really does smile a lot!) have been when he has beaten his medically trained colleagues to an important conclusion on a clinical aspect of the research. – *Dr. Harold Swartz, Dartmouth University*

70th Birthday of Carlo Corvaja

TAKEN BY MARINA BRUSTOLON

Carlo Corvaja was born in Venice on the St. Elena island. He still recalls dearly the countless plunges in the lagoon and the breathtaking marine explorations, where St. Elena appears as the island of Neverland. It is a great privilege for a child to be raised in Venice, but for one as lively as Carlo it was simply fabulous. His love affair with the lagoon and the sea led him later to take up sailing, a sport in which he excelled. His sailing career culminated in 1963, when he qualified in the Italian Championship on a snipe.

I have no doubt that he did not leave Venice willingly, but regrettably the University of Padova was on terra firma, and there young Carlo studied Chemistry and obtained his undergraduate degree in 1962. During his studies he became acquainted with the world of free radicals and paramagnetic species; his thesis work focussed on radical ions of carbonyl compounds. It must have been at this stage that his curious and bright mind was captivated forever by science. He was supervised by Giovanni Giacometti who championed, first in Italy, the use of EPR in physical chemistry research, and who found in the late Pier Luigi Nordio and Carlo his most gifted students, and later outstanding scientific collaborators. In the group of Giovanni there was also a brilliant young researcher, Maria Vittoria Pavan, who was involved in theoretical interpretation of hyperfine couplings in charged radicals. She had just the time of publishing a seminal paper [G. Giacometti, P. L. Nordio and M. V. Pavan, Theor. Chim. Acta, 1963, 1, 404] before becoming the wife of Carlo and giving birth to a pair of twins (Pietro and Roberto, today a mathematician and an engineer, respectively), later followed by other two brothers (Fabio and Giovanni, now a lawyer and a goldsmith, respectively).

Collaboration with Hanns Fischer brought Carlo to Darmstadt, and coming back to Padova he set up an apparatus for analysing unstable radicals in a flow system. In 1968 Carlo spent one year in Nijmegen, Holland, in the laboratory of Edgar De Boer, carrying out NMR studies on radical ions and ion pairs in solution. Back to Padova, he started an intense research activity in the field of EPR of ion pairs in solution, together with two young graduate students, the late Luigi Pasimeni and myself. For a few years the main activity of the group was glass-blowing and in that nobody could possibly even dream of competing with Carlo, inspired by the great masters of the Murano tradition!

In those years Carlo was rushing ahead through his academic career, and in 1976 he got his full professorship in Padova, where he read physical chemistry, molecular spectroscopy and theoretical chemistry to Chemistry undergraduate and graduate students. In his teaching style he put a particular emphasis on the intuitive physical model lying behind the mathematical formalisms; this reflects his more general approach of going always to the core of problems.

In his scientific activity, Carlo has always strived to try new methods and approaches in the field of paramagnetic resonance. By taking advantage of the limited technical support he could get from the chemistry area in Padova he succeeded in assembling novel instrumentation, which enabled our laboratory to perform cutting-edge research. On his craft skills I can speak out of personal experience; in 1975 I did one-year post-doc in Sheffield under the supervision of Neil Atherton to familiarize myself with ENDOR techniques and once returned, I was helped by Carlo, who gave a fundamental contribution in building a home-made ENDOR machine for our laboratory.

In the 1980s, Carlo managed to put together a time-resolved EPR spectrometer and thus lay the foundations for a series of fruitful experiments, carried out in collaboration with Luigi Pasimeni, in the field of photoexcited polarized triplet states. This technique, together with ODMR, was then applied to charge transfer molecular crystals; these experiments constitute the core of more than 30 research papers. Luigi Pasimeni died tragically in 2001. His presence in the department has been sorely missed.

At the end of 1990s Carlo opened a new avenue of investigation focussing on paramagnetic fullerene derivatives in ground and excited states. For his pivotal studies on the interaction between free radicals with excited triplet states, he shared with Prof. Seigo Yamauchi (Sendai, Japan) the 2001 Silver Medal of the International EPR/ESR Society. He was also awarded the Gold Medal of the Magnetic Resonance Group of the Italian Chemical Society, which celebrates his more than 200 contributions to the study of paramagnetic systems, many of which are the result of collaborations with scientists from different countries.

One of the most striking paradoxes of the Italian academic system is that it is extremely difficult for researchers to progress in their career anywhere else but where they have been formed, unlike what is seen in other countries, where high mobility of researchers is a prerequisite to a successful scientific career. Consequently, some of Carlo's former students are still in the same Department of Chemical Sciences in Padova, researching in the various fields of EPR spectroscopy and derived applications. Such a high density of scientists working elbow-to-elbow on similar topics is sometimes the cause of problems, but today it gives Anna Lisa Maniero, Antonio Toffoletti, Lorenzo Franco, Antonio Barbon, Fosca Conti, Marco Ruzzi, Alfonso Zoleo and myself the occasion to wish him together all the best, and to thank him for conveying to us the fruits of his imaginative mind and his love of science.

Carlo's old supervisor Giovanni Giacometti is also still as Emeritus in the Department and is still keen on what goes on in the group on Biophysics and Photosynthesis, born in the 1980s, and together with Donatella Carbonera, Marilena Di Valentin, Giancarlo Agostini and Stefano Ceola unite to the good wishes for Carlo.

Marina Brustolon

65th Birthday of Hans Wolfgang Spiess

On October 14, 2007 Hans Wolfgang Spiess turned 65. Although he is best known for his contributions to solid-state NMR spectroscopy, his ideas have also influenced the field of EPR spectroscopy, in particular with respect to studies of structure and dynamics of partially ordered solids. Hans Spiess studied chemistry at University of Frankfurt and joined the group of Hermann Hartmann for his diploma and PhD theses on solid-state NMR of single crystals. During a postdoctoral research stay in Florida he first turned to the problem of averaging of anisotropic parameters by motional processes. After coming back from the USA in 1970 he joined the group of Karl Hausser at Max Planck Institute for Medical Research in Heidelberg, which was probably the most active magnetic resonance group in Germany at that time. In 1975, he moved to the newly forming group of Hans Sillescu in Mainz where he finally decided to focus on the application field of polymers and other partially ordered solids. His 1978 paper on determination of orientational distributions in such materials is concerned with both NMR and EPR line shapes [1]. After a short term as a Chair for Macromolecular Chemistry in Bayreuth in 1983 Hans Spiess returned to Mainz where he was appointed director of the department of Polymer Spectroscopy at the newly created Max Planck Institute for Polymer Research.

For almost a decade he forgot about our beloved EPR spectroscopy and concentrated on solid-state NMR, mainly on deuterons. At some point he must have realized that some problems could be treated more easily by EPR. When he received the prestigious Leibniz prize of Deutsche Forschungsgemeinschaft in 1988, he invested a substantial part of the prize money for a commercial pulsed EPR spectrometer. However, he was not satisfied with just using the in-built capabilities of such instrumentation. Based on a continuous-wave spectrometer his newly formed EPR subgroup built imaging equipment and soon published one of the first application examples of EPR imaging in materials science [2]. The study provided insight into kinetic aspects of UV irradiation of photoresists, the types of formed radicals, and their spatial distribution.

The first work on pulsed EPR also depended on homebuilt hardware. Paralleling developments of two-dimensional Fourier transform EPR experiments in Jack Freed's group, the Spiess group observed magnetization transfer of nitroxides by different processes using a fast field-step electron-electron double resonance (ELDOR) setup [3]. This study nicely exemplifies the hallmark of Hans Spiess' research in both NMR and EPR: a sample of high current interest, in this case a liquid-crystalline side-group polymer, new magnetic resonance methodology, and a quantitative interpretation of the results in terms of soft-matter physics.

The research environment that Hans Spiess maintained in his group was ideal for cross-fertilization between NMR and EPR. One of the most daring projects that resulted from such thinking was the introduction of magic-angle spinning EPR in 1994 [4], based on the (slow) magic angle turning experiment in solid-state NMR. Due to technical limitations this experiment is not yet widely applicable, but we may well see its renaissance in the future.

The use of nitroxide probes and labels to elucidate structural dynamics in soft matter became one of the main directions of his EPR research. Work in this direction started with a careful study of differences between a spin probe and a chain-end label in polystyrene [5]. When I joined his group I was impressed by the 'spectral eye for nitroxides' that Hans Spiess had developed. With a short glance he could see the story told by a series of nitroxide spectra. Later I found that this capability does not only depend on knowing the typical features of such line shapes but also on a broad knowledge about possible dynamical processes in the sample.

From 1997 to 2006 I had the honour – and pleasure – to take responsibility for the EPR group of Hans Spiess' polymer spectroscopy department. His stimulating presence and broad overview of soft-matter physics had a strong influence on my own research profile, which I developed during this time. The similarities and differences between NMR and EPR were often a focal point of our discussions [6].

During his career Hans Spiess collected a number of prestigious awards, such as the Liebig medal of GDCh and the AMPERE prize in 2002, the prize of the Society of Polymer Science and Technology Japan in 2003, and the Walter-Nernst medal of Bunsengesellschaft für Physikalische Chemie in 2005. He also acted as a representative for science in general, as a member of the federal German Science Council 1999–2005, and for our research field as a president of Groupement AMPERE from 2000–2006.

His 65th birthday is not an occasion for him to retire. He continues research, also in the field of EPR spectroscopy, where Dariush Hinderberger has taken over as a project leader. We wish Hans' Spiess all the best and many new ideas in both NMR and EPR spectroscopy.

Gunnar Jeschke,
University of Konstanz, Germany

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Dieter Schmalbein: 65th Birthday, but no Stop in Sight

On October 22th, 2007 Dieter Schmalbein has celebrated his 65th birthday, believe it or not. Among his long-standing members of staff and friends everyone is getting gray hair, except one, yes, Dieter Schmalbein. Not only is his hair full of strength, his whole personality as Managing Director of Bruker BioSpin GmbH and Bruker Optik GmbH is full of enthusiasm, foresight, thrust and spirit. He has boundless energy and that is good because he works hard all the time.

During his celebration party in the EPR division of Bruker, he gave a very appealing presentation of his life, starting from the very beginning, i.e., his childhood, up to the present. 'Up to now,' one should add, because he will continue as Managing Director and moving force responsible for the EPR division.

Attending the many hundreds of conferences for bringing Bruker EPR to his customers, Dieter enjoys his passion, taking pictures and videos, boosted now by the digital era. Surely you will meet him and his charming wife Cornelia during one or another conference in the future.

We are thankful to Dieter Schmalbein and wish him all the best.

Art Heiss, Andreas Kamlowski,
Diether C. Maier, Peter Höfer

40 Years of Bruker (UK)

The fortieth meeting of the ESR Group in Oxford in March 2007 had something of a special resonance with Bruker. Indeed you could even say there was a double resonance, since 2007 marks forty years of trading for Bruker in the UK, and the very first business address was in the city of Oxford.

John Anthony Deegan, or Tony Deegan as he liked to be called, was in the mid 1960's a salesman for ELGA Process Water. With a graduate chemist background, Tony was above all an entrepreneur. He had a very unconventional approach to almost everything and he hated procedures. He also possessed a real flair for salesmanship. Looking for a new challenge, whilst he was on a sales trip to Germany in 1967 he managed to get an appointment with Professor Günther Laukien, the owner of the fledgling Bruker-Physik AG.

Bruker had been founded in Germany in 1960 and at that time was producing a range of special electromagnets and power supplies, and was pioneering the commercial supply of high-power NMR spectrometers. With the acquisition of the assets, staff and know-how of the bankrupt Trub-Taüber business and the setting up of Spectrospin AG in Switzerland in 1965, Bruker had moved into high-resolution NMR, and at about the same time had started the design and development of CW-EPR spectrometers.

Tony Deegan saw some considerable sales potential for Bruker's new technology. So confident was he of success that he offered to work for no salary, and to take only a commission on what he sold. In the exchanges that followed their first meeting he persuaded 'the Professor' to agree to the founding of a UK company, Bruker Spectrospin Limited, on 30 November 1967.

The office was a spare bedroom in a small apartment in Woodstock Close, Oxford. This location was obviously very convenient for making personal contact with the scientists at Oxford University, but with the disjointed road and rail network at the time it was not at all suitable for visiting the wider UK scientific community. Nevertheless, early success came in 1968 with the first sales, 90 MHz high-power pulsed NMR spectrometers to the University of Salford and to the University of Leeds. With some great forethought, bearing in mind that the motorway network in the UK was at that time still largely un-

developed, the office was moved in 1969 from Oxford to the eastern side of the City of Coventry, again to a private address. After a couple of years of working from very limited accommodation, Bruker in the UK doubled in size in 1971 when it moved to a much larger private house with not one, but two spare bedrooms.

The beginning of the seventies marked the start of major growth for Bruker worldwide. The introduction of commercial superconducting NMR spectrometers, solid-state electronics and compact electro-magnets at a time of growth in scientific research projects led to obvious commercial benefits. Not surprisingly, with the hiring of an Applications Scientist and a Service Engineer to support the increasing business in the UK, the days of the two spare bedroom enterprise were numbered. And so began a long year search for a suitable location to build a UK Bruker office. Tempted by Government financial assistance in the form of Regional Development Grants, many greenfield sites, development sites and all manner of building conversions all over England, Scotland and Wales were considered; all of them rejected finally for one simple reason – access to customers.

Whilst this search was going on, the UK office was "temporarily" re-located to a rented industrial unit in Coventry, in this instance temporarily lasting for 14 years. It was at about this time that the local office celebrated its first EPR sales success, with the delivery of an ER-420 instrument to Trinity College, Dublin.

The extra space did bring much needed relief and afforded the opportunity to install an NMR system on permanent demonstration, with EPR systems being brought in and set

up as required for training purposes or for seminars. Being located in a small industrial neighbourhood was not altogether without problems though, and the viability of the enterprise was severely tested when a new neighbour with a very good business in RF plastic welding moved in next door. As luck would have it, the RF frequency used for plastic welding had very strong harmonics at 90 MHz, so in order to run to run a decent NMR demonstration it was necessary for the Bruker Applications Scientist to go next door and negotiate with the factory manager for a convenient welding-free time slot. Notwithstanding such irritations, staff numbers grew in sales and service, and by the end of the 1980's the UK team was 22 strong.

When the Wickman Machine Tool company in Coventry, famous worldwide for its multi-spindle automatic lathes and milling machines, was bought up in 1984 a large parcel of land adjoining their factory was put on the market. Located just 2 km from Bruker's then office it looked to be the ideal site on which to erect the new building that by now was desperately needed. It took two years to negotiate a deal, and then another three years to design and build what is now the headquarters of all of Bruker's UK businesses. Moving from a cramped and inadequate 300 m^2 industrial unit into 4,000 m^2 of purpose-built office and laboratory space seemed then like a dream come true.

The UK market has always been hugely important for Bruker. Notwithstanding the varying economic difficulties faced by some scientific institutions, the quality of UK scientific research is consistently high, with a continuing demand for specialised instrumentation. From the start of the supercon-NMR days, the relatively high cost of an NMR spectrometer was something that scientists had come to accept. Not so though with EPR, where a CW system could be had for a fraction of that price. Therefore, many eyebrows were raised at the price tag when

the first commercial FT-EPR system was announced, but the UK scientific community recognised at an early stage the potential of FT-EPR by funding one of the very first Bruker pulsed EPR system to be delivered, to University College, London in 1989.

The expertise and additional resources gained through relocating the magnetic resonance imaging business of Bruker's subsidiary Oxford Research Systems from Oxford to Coventry, opened up the possibility for a new venture for the Coventry site. Two applications scientists, one from an imaging background and one from an EPR background, and an RF engineer found themselves sitting together one day around the lunch table. Not given to idle chat about football or the weather, the British staples of at-table conversation, the seed of a new idea was sown and over a few months was germinated into a major plan for a new product development. Working with R&D engineers in the factory in Germany, and in collaboration with a UK university, the L-band EPR imaging spectrometer was designed and developed in Coventry, prior to its transfer to Germany for manufacture.

Bruker's initial core business of magnetic resonance was complemented in the 1970's by the addition of FTIR and FT-MS, together with a number of other analytical technologies. The UK office embraced all of these additional products by investing in new people to promote and support the widening product portfolio. At the same time, the changing attitudes in the UK towards service support shown by both industry and academia, and the closing of local lab workshops presented the local Bruker organisation with an opportunity to at first assist with and then largely take over the instrument service support role. With a huge and ongoing investment in people, training, equipment and systems the UK office established for itself over the next ten or so years an enviable reputation for technical and service support.

The acquisition of the analytical x-ray business from Siemens in 1997 marked the start of a significant new phase in the overall business development plan. Coincident with the establishment of a separate Bruker x-ray business in Karlsruhe, Germany, the well-established ex-Siemens x-ray team in Manchester was re-formed as Bruker-AXS and relocated to Congleton in Cheshire. This then became the model for a new global Bruker template for business structure. As in many other countries, the UK organisation was restructured into individual technology specific businesses, each with its own dedicated sales and scientific and support teams. Sharing common administrative resources the five businesses known as Bruker BioSpin, Bruker BioSpin MRI, Bruker AXS, Bruker Daltonics and Bruker Optics together represent the complete range of analytical and process control products.

Now, forty years after its birth, Bruker in the UK approaches middle age. Its workforce has grown from one to over one hundred people, but it has not become bloated. Like many of its markets and product technologies it has matured, but it has not lost its youthfulness, verve or sense of adventure. The founding employee was unconventional, was an entrepreneur and liked to do things by the seat of his pants. He never lost sight, though, of the fact that it was the customer who paid his wages, and that the customer was the most important person in the Bruker organisation. Forty years later that's as true to day as it was then.

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ESE in Biology
Bill Mims' Reminiscences*

In this final instalment of notes by Bill Mims, written after he had retired from the Bell Telephone Laboratories more than 20 years ago, we find the fascinating, on-going story of the development of pulsed EPR techniques. It may come as a bit of a surprise to learn that Mims initially regarded the nuclear modulations as something of a nuisance and also that his colleague Bill Blumberg lost interest in pulsed experiments. The story shows that science is not a simple linear process, new innovations are not always immediately embraced and illustrates a human side that would never have appeared in published papers. An early issue was to do with whether the modulations observed in pulsed experiments would supplant ENDOR particularly with reference to biological systems. The classic example of the copper protein stellacyanin makes for interesting reading. The section on three-pulse experiments describes the genesis of what we now know as Mims ENDOR. Ever the physicist, Mims wanted to achieve more than just empirical relationships and to understand the underlying physics, which he succeeded in doing. Mims also explains his early forays into use of the Fourier Transform, something that we really do take for granted these days. Without easy access to cheap and fast computing, Mims lets us think through the issues he faced, in particular, how he dealt with the dead-time problem. Without these insights into the data analysis, backed up by his physicist's understanding of what was really going on, the progress we see today would not have been possible.

To sum up, Mims' notes provide a unique historical account of the evolution of pulsed EPR methods that are now standard practice in many laboratories around the world. It is good that these notes were not lost but have been allowed to see the light of day. They provide a very nice historical background to the much more technical issues that subsequently formed the basis of books by Dikanov and Tsvetkov and, more recently, the classic by Gunnar Jeschke and late Arthur Schweiger.

It is best, however, to let these reminiscences tell their own story.

* In this issue we have combined Parts II and III we spoke about in the introduction to the first instalment of Mims' notes (newsletter 17/1, pp. 10–12).

The Nuclear Modulation Effect

The vastly improved signal to noise factor, and the wide variety of samples that were now being studied in quick succession with the new cavity, drew attention to the possibility of making some use of the 'nuclear modulation effect' by attempting to interpret the characteristic patterns which it imposed on the echo decay envelopes. The nuclear modulation effect, a phenomenon observed in almost all electron spin echo experiments, had, since its discovery in 1961, been more of a nuisance than anything else.

For most purposes the ENDOR double resonance method invented by George Feher at Bell Labs was easier to apply, and, at the low radio frequency end, where the conventional ENDOR method ran into difficulties, an electron spin echo ENDOR method could be used with less trouble. The theory of the effect was worked out in a paper by Hahn, Kaplan, and myself (Kaplan dropped out of the final paper to make way for one of Hahn's graduate students), and several studies were performed subsequently in which nuclear modulation patterns were successfully simulated from ENDOR data. But the effect remained a mere curiosity.

The conventional ENDOR method was extended to the study of biological materials by Charles Scholes (a Feher post-doctoral student), but it was clearly much more difficult to apply in this context, one reason being that single crystal samples are so rarely available.

This, and the steady improvement in data collection and Fourier transform techniques, raised once again the question of whether the nuclear modulation effect might not provide a viable alternative. In 1972 I made an attempt to do this with Bill Blumberg, who, discouraged by the practical difficulties of this approach, finally lost interest. I myself became convinced that this method of obtaining electron nuclear interaction data was still as unpromising as ever, and regretted the effort put into a long Phys. Rev. paper which I had simultaneously prepared on the general theory of the effect for two and three pulse echo sequences and for arbitrary nuclear spin. Reprints of this paper remained virtually untouched in my filing cabinet for four years until 1976, when they began to dwindle rapidly.

In returning to the nuclear modulation effect (towards the end of the heme compound electric field effect series), my colleague Jack Peisach suggested that we should learn from earlier abortive attempts, and avoid trying to extract ENDOR frequencies until such time as we had been able to accumulate a library of modulation envelopes for samples of different kinds. By comparing these we should at least be able to extract some useful biological information, and we might, as in the case of the electric field effect experiments, be able to think of better ways of doing the experiments as time went on. This, I should point out, is not the way I should have approached

the problem if left to myself, but it was the key to our eventual success.

Stellacyanin

These echo envelope tracings yielded their first scientifically useful result with observations made on the copper containing protein stellacyanin. In addition to the high frequency modulation patterns found in all these tracings, which was attributable to hydrogen, there was a prominent low frequency component which, in the absence of any other possible cause, had to be due to nitrogen nuclei. This was not in itself a novelty, since low frequency modulation components (with different patterns) had already been observed in tracings obtained for the heme compounds. The novelty consisted in the fact that none of the copper complexes previously tested (as a by-product of some studies on the electric field effect in nonbiological copper complexes) had shown any low frequency pattern at all. Their absence in all these cases had been understandable since copper was known to form a partially covalent bond with nitrogen, resulting in a strong contact I·S type interaction which split copper resonance lines to a sufficient extent to be visible in conventional EPR. The ENDOR frequencies of copper bound directly to nitrogen nuclei would therefore be much higher than the frequencies observed, besides which, the theory of the modulation effect predicted the absence of modulation in cases where there was a strong I·S coupling. The indications in the case of stellacyanin were therefore for a more weakly coupled nitrogen, not directly coordinated with the copper ion.

Since stellacyanin is a protein, the coordinating ligand responsible for the pattern had to belong to one of the amino acids, which might be identified by trying out a series of model compounds. The second of those to be tried gave an echo envelope pattern virtually indistinguishable from that obtained with stellacyanin, and thus identified the ligand as the imidazole side chain of the amino acid histidine.

As on many subsequent occasions, there were then requests coming in from other laboratories, asking that the same experiment should be performed on some similar protein of particular interest to the research group in question, in order to see if the same structural feature occurred there too. Not all of the experiments done in response to these demands yielded identical patterns, but the results, which generally concerned bonding features due to nitrogen belonging to an imidazole ligand, were sometimes useful as a source of structural detail. However, only in one case, that of superoxide disnutase, where there is secondary bonding of the imidazole to zinc, did these measurements lead to a new set of experiments in our laboratory.

Explaining the Result

A more direct concern to myself, as a physicist, was to go beyond this 'fingerprint' approach and find an explanation for this pattern in terms of physical parameters, such as the electron-nuclear coupling, and the nitrogen nuclear quadrupole interaction. Direct measurement of the nitrogen frequencies from modulation tracings did not appear to be possible, because of the short duration of the echo envelope (about 2.5 microseconds on account of hydrogen in the environment). Fourier transformation of the envelope was even less promising, because, in addition to the problem of the waveform's short duration there was the problem of dealing with the relatively long dead time at the start (corresponding to the time taken for the high power microwave pulse in the resonant cavity to decay to thermal noise levels). It began to seem as if the double resonance ENDOR approach would have to be adopted after all, in order to extract the nitrogen frequencies and undertake a simulation. This was at the time not an unreasonable conclusion, since all previous interpretations of a nuclear modulation pattern in single crystal samples had ultimately depended on a set of ENDOR measurements. However, in this particular case it was clear that the Feher method would not work, because of the weakness of the electron nuclear coupling parameter and the low values of the radio frequencies that were to be expected. So preparations were made to set up the electron spin echo ENDOR system which had been shown ten years earlier (in calcium tungstate) to be good down to 0.2 MHz.

Three Pulse Experiments

In an indirect manner this decision lead to a solution of the problem, although the ENDOR experiment itself was never done. In spin echo ENDOR experiments of this low frequency type a radio frequency pulse is applied between pulses II and III of a three pulse echo sequence. The first requirement is therefore that it should be possible to separate pulses II and III by a time interval long enough for the radiofrequency pulse to produce its effect, without losing the stimulated echo (Erwin Hahn's name for the echo that follows the third microwave pulse) due to various, imprecisely understood relaxation mechanisms. A beginning was therefore made by checking the behaviour of various hydrogen containing samples, including any copper proteins currently available, for three pulse stimulated echo decay times. The times observed were reassuring. For reasonable values of the pulse I to pulse II times (these times also condition the stimulated echo decay rate) up to 40 microseconds could be allowed between microwave pulses II and III without losing the signal. This was a welcome surprise after a long series of two pulse echo observations which had been limited to 2.5 microseconds at the maximum. More surprising, however, was the appearance of what seemed to be an ineXplicable artifact. The stimulated echo was showing large oscillations in amplitude as a function of the pulse II to pulse III time for intervals beyond 10 microseconds. The theory of envelope modulation formulated several years earlier in 1972 did indeed predict a modulation effect in the three pulse mode, and such effects had been noted, though not studied in detail, in some single crystal samples even before 1972. But it seemed virtually impossible that a lengthy pattern, containing many low frequency cycles, should be obtained with a non-crystalline, frozen solution sample, especially one involving nitrogen nuclei with their anticipated nuclear quadrupole interaction and the random orientation of the complexes.

The puzzle might perhaps have been solved in several different ways. The observation of a long modulation pattern, corresponding to a relatively narrow ENDOR line (by the standards of non-crystalline protein samples) at first lent additional impetus to the preparations for spin echo ENDOR experiments. The puzzle might also have yielded to a lengthy series of computer simulations, with many trial values of the parameters and appropriate spherical averaging of results, although it is difficult to see how this could have succeeded with acceptable consumption of computer time (then limited by the department budget) unless the nature of the answer had been foreseen in some detail.

In the event, the solution was found by substituting the nitrogen 15 isotope for nitrogen 14 in one of the model compounds. Without any quadrupolar interaction to complicate the picture the answer soon became apparent. By a fortunate accident, the applied magnetic field in these X-band microwave experiments was of the right magnitude to cancel the weak contact 1.5 interaction between the indirectly coordinated nitrogen and the copper. So, for one of the two possible electron spin orientations we were reproducing zero field quadru-

polar resonance conditions for the nitrogen, and the ENDOR resonance was as well resolved as it would have been in an NMR zero field quadrupolar experiment.

As a postscript to this research episode it may be noted that some years later a postdoctoral student in the University of Michigan, who was considering the relative merits of the echo envelope modulation method and traditional ENDOR, realised that the narrow nitrogen line observed in our experiment could not have been seen at all by ENDOR. Just as an ENDOR line can sometimes be enhanced by the electron nuclear interaction, so it can also be reduced in strength. Under the particular conditions that obtained in our experiment, the radiofrequency signal applied in an ENDOR experiment would have been precisely screened out by the 1.5 electron nuclear interaction.

It may also be pertinent to explain who why three pulse experiments had not been initiated much sooner, in view of the fact that it was known from theory and from earlier electron spin echo experiments that there would be modulation effects to observe. One reason lay in the considerably greater time that would have been needed for each set of measurements. Not one, but a series of three pulse tracings, at various fixed settings of the pulse I to pulse II interval, would have had to be recorded, since theory predicted that certain modulation components would be suppressed at specific settings of the pulse I to pulse II time. An even more compelling reason for avoiding three pulse experiments had been the unavoidable increase in the dead time interval which had to elapse after the pulse sequence before envelope recording could begin. This, and a further complication due to the appearance of unwanted echoes, such as the two pulse echoes generated by pulses I and III and pulses II and III, and a 'refocusing echo' for which one of the unwanted two pulse echoes acted as source. Miscellaneous measurements on protonated and deuterated materials, made in previous years, had borne all this out. The accidental cancellation of the externally applied magnetic field (the Zeeman field) by the electron nuclear contact interaction changed the outlook for three pulse experiments, especially when later on ways were found to suppress most of the 'unwanted' echoes, or at least to ensure that they did not appear as glitches in the three pulse envelope tracing. With these technical improvements, and with the realization that the 'suppression effect' occurring for certain settings of the pulse I to pulse II time could sometimes be used to identify very broad ENDOR lines that would not readily show up in the envelope itself, the three pulse method has come to preponderate as the method of choice in nuclear modulation experiments, even where nitrogen 14 quadrupolar effects are not involved. The substantially longer duration of the three pulse echo envelope often makes up for the greater complexity of the experiment.

Fourier Transformation

Fourier transformation of the echo envelope was, as mentioned earlier, attempted soon after the discovery of the effect in 1963, since when it had been intermittently suggested by various persons as an obvious complement to the recording of the envelope itself. However, the first scientifically useful application of the idea-useful since it revealed a nuclear interaction that had not been detected by simple inspection of the time waveform-was made by Toru Shimizu, a Japanese post-doctoral visitor, in 1977. This was the Fourier transform of the two pulse echo envelope obtained with a neodymium ATP (adenosine triphosphate) complex, which was being used as a model in some experiments involving the creatine kinase plus metal ion substrate system. In addition to showing hydrogen and phosphorus modulations, which were apparent in the time wave, the Fourier transform showed coordination by a sodium nucleus originating in the Na ATP salt used to prepare the sample. (This revelation of possible impurity effects, disconcerting perhaps to the biological community, was published in J. Chem. Phys. and not in the biological literature).

The Fourier transformation was done in two ways. One result was obtained by taking the square root of the Fourier power transform of the actual recorded trace, smoothing out where possible the artifacts introduced by the absence of the dead time portion at the start of the time wave. A second spectrum was obtained by taking the Fourier cosine transform of a reconstructed curve, in which the initial portion had been sketched in freehand so as to appear as reasonably consistent with the subsequent recorded portion as possible. (By theory, all the modulation components are cosine functions as referred to time zero at pulse I of the sequence.) This reconstruction procedure yielded a far clearer picture, and numerous subsequent attempts were made to automate it by a computer program. Recently, in 1984, a group at Delft in the Netherlands has succeeded in overcoming the dead time artifact by applying a curve fitting algorithm. This approach had been strongly urged on me by Richard Hamming at Bell labs, but at that stage in the development of electronic data processing, it appeared to be too expensive in computer time, and also unsuitable for experiments in which a frequency spectrum was required immediately and not the morning after. In the early 1980s computer aided reconstruction followed by Fourier cosine transformation seemed the most practical and economic approach to the dead time problem. However, with the rapid increase in computer speed, as predicted by Moore's law, and the concomitant reduction in expense, it is likely that curve fitting will become the standard method of analyzing echo envelope recordings in future. Improvements in the technique of three pulse envelope spectroscopy, with Fourier transformation, made it possible to interpret earlier observations on the heme compounds, and to extend these experiments with a new study of cytochrome P450. Published results also attracted the attention of other laboratories that began to set up facilities for themselves. This was a turning point in the work in Bell labs, where it became apparent that the field was rapidly becoming too wide to be serviced by our very limited resources. I suggested that new electron spin echo systems should be designed to cover other microwave ranges, outside X-band, so as to provide complementary data, as for instance in the case of nitrogen coupling, where different values of the Zeeman field might be needed to achieve the cancellation of I·S contact interaction and yield the kind of narrow line spectrum we had observed in stellacyanin. These suggestions mostly fell on deaf ears. University researchers, dependent on contract support, needed to be absolutely sure of success, and this could most reliably be achieved by making a near copy of what we had already developed at Bell labs. In such an environment it seem unlikely that the kind of work described here could ever have been done.

Observations with Deuterium

It would be tedious to list all the other enzyme systems that were examined in our own labs-samples prepared by Jack Peisach at the Einstein College of Medicine and by researchers at other university laboratories. However, one item may be worth mentioning since it illustrates the versatility of the electron spin echo method. It had been noted in some experiments on the ferredoxins (complexes containing iron and occurring in a variety of proteins) that, if the samples were exposed to D20 for varying periods of time, a deuterium modulation could be ob-

served in the two pulse envelope. Since the Fe atoms are already fully coordinated and are enclosed in a protein environment this pattern yielded evidence for the unfolding of the protein under selected pH and oxidoreductive conditions to admit the D20.

Professor Orme-Johnson (now at MIT) who had inspired these experiments made repeated requests for a numerical characterization of these results, but it did not at first seem possible to go this far, both because of uncertainties introduced by the dead time, and because of a general lack of experience in handling this sort of problem in simpler situations. The deuterium nucleus has a small quadrupolar moment, and the resulting shifts in the nuclear resonance frequency are manifested in the echo envelope as an accelerated decay pattern (corresponding to a broadening of the associated ENDOR line in a non-crystalline sample). This makes it difficult to extrapolate to zero time, and thus estimate the depth of the modulation as it would be in the absence of phase memory decay and quadrupolar effects. If it were not for this problem then the distance to the deuterium could have been inferred from theory, which predicts a modulation depth proportional to 1/r6.

The problem of quantifying modulation patterns due to deuterium seemed one that was likely to resurface in future work and therefore to justify a special effort. Theory predicts that the modulation pattern due to several nuclei is given by the product of the modulation patterns for each nucleus considered separately. So, as a first step the modulation pattern with a deuterated ferredoxin was divided by the envelope obtained with a non-deuterated one. This eliminated much of the modulation due to the normal hydrogen in the protein. (Actually, since some fraction of the normal hydrogen had been replaced by deuterium this procedure left a small artifact, i.e. the reciprocal of the pattern due to the substituted hydrogen. But this was a minor problem since only a small part of the hydrogen was involved.) Alternatively, a three pulse experiment could be performed with the pulse I to pulse II interval set so as to suppress hydrogen modulation.

The 'cleaned up' pattern was then compared with computer simulations for a set of deuterium coordinated complexes. In order to make these simulations it was necessary to take account of deuterium line broadening due to the deuterium quadrupole moment. Since the corresponding quadrupole frequency was small in relation to the deuterium nuclear resonance frequency (about 2 MHz for our values of Zeeman field) this was inferred by taking a spherical average over all orientations. This procedure could not be rigorously justified, but an extensive series of more careful simulations for a deuterium complex, made by the Novosibirsk group, showed that the error would be minimal, provided that the measurements of modulation depth were made on the first deuterium cycle to be observed in the envelope, and not on the envelope as a whole.

Unfinished Work

With this much accomplished how many other avenues of exploration had it been necessary to pass by? One was the application of spin echo ENDOR techniques to biological materials. A major obstacle here was the attitude of the immediate Bell labs management that had convinced itself that Endor of any variety was an old outdated technique, invented long ago by Feher when he worked at the labs, and not a fit subject for current research. I feared that if, I were to insist, the whole electron spin echo project might be terminated by edict. (The days when Rudolf Kompfner had been my lab director were long past.)

Just as the echo method can be used to find the distance between a paramagnetic ion and a neighboring nucleus, so it might be used to find the distance between two paramagnetic ions, for example an enzymatic active site and a spin label free radical. This can be thought of as a variant of the spin echo ENDOR technique, where the flipping of a nuclear spin speeds up or slows down the steady precession of a nearby electron. But in this case the magnetic moment of the second electron spin is much larger than that of any nucleus, and the effect would be detectable at a much greater range-for example 35 Angstroms. The technical problem would be to introduce two microwave frequencies into the resonant cavity, one frequency for each of the electron spins involved. Some work along these lines by the Novosibirsk group, employing only one microwave frequency had demonstrated the practicality of this type of experiment, which would presumably go by the name of spin echo ELDOR, using an acronym borrowed from analogous experiments in continuous wave EPR. A preliminary model of a two frequency resonant cavity was made in the Bell Labs workshop, but no further tests were made.

Finally I have to express regret that electric field effect experiments were never made on a single crystal protein sample. The effects are there to be measured, and the requisite high electric field steps can be applied to protein samples without causing electrical breakdown, as shown by experiments on frozen solution samples. Each experiment would take a considerable time to perform, since the metal ion sites in proteins are characterized by a low symmetry and could yield up to eighteen independent parameters, but a problem of this magnitude was already dealt with in 1968 in a study of anomalous charge compensation sites in a single crystal of doped calcium tungstate. Perhaps the major problem here would not be the technical one, but that of finding a theoretical chemist both willing and able to interpret the results and explain them in terms of the chemical bonding of metal ions in proteins.

PROS AND CONS OF PULSE DIPOLAR ESR: DQC & DEER

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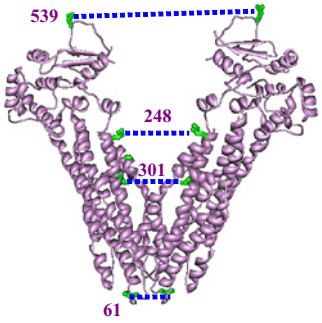

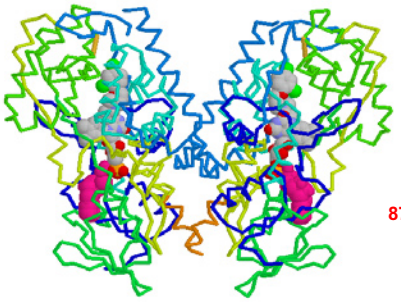

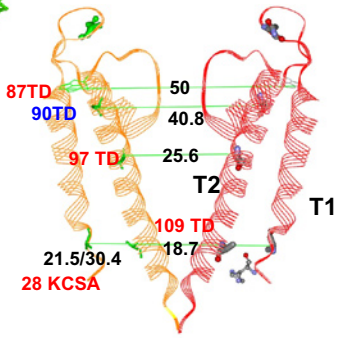

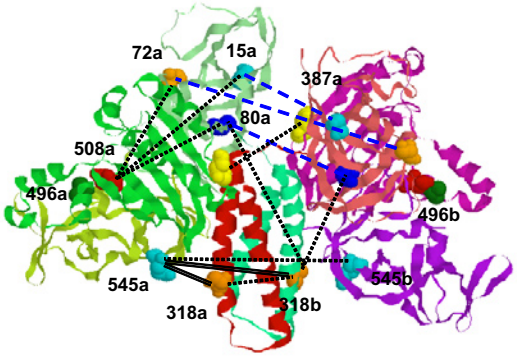

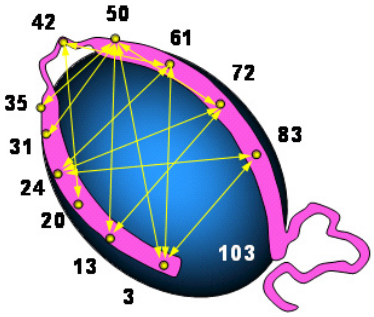

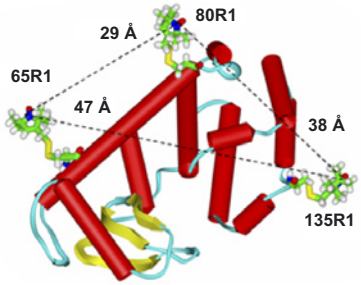

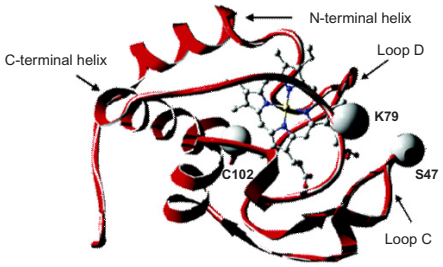

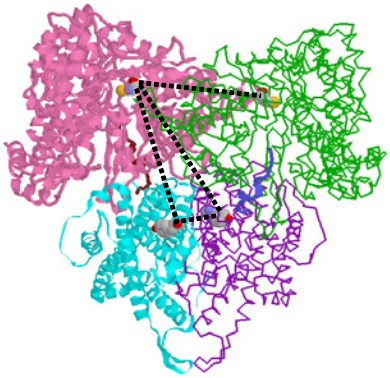

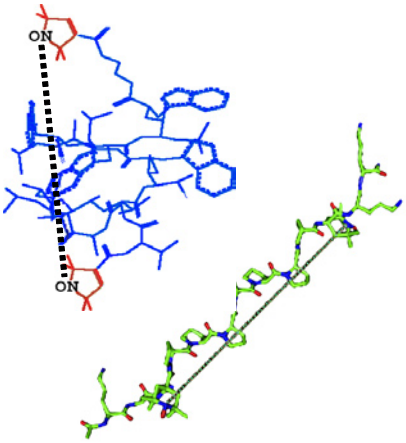

Front Cover. A collection of proteins and peptides studied at ACERT. The collection illustrates various points of PDS associated with each system studied.
Upper row. Left: Multi-drug ABC-transporter, MsbA: dimeric; reconstituted in membranes and detergent micelles; several functional states studied. ***Center***: Monoamine oxidase, MAO-A (and MAO-B's from different organisms): oligomerization state in native membranes; reconstitution in detergent micelles and native membranes; spin-labeling with spin-labeled inhibitors and radical cofactors. ***Right***: KcsA (Also MscL, and KvAP): tetramer membrane channel; tandem dimers and tetramers; liposomes; multiple distances in oligomeric state. (unpublished data).
Middle row. Left: Histidine kinase, CheA complex with CheW: triangulation; protein complex; oligomeric; heterodimers and heterodimeric complexes. ***Center***: α-Synuclein: polymorphic (unstructured and highly structured); soluble and surface-bound to micelles and liposomes; MTSSL labeling; rigid-body modeling. ***Right***: T4-lysozyme: multiple distances; deuterated solvents; triangulation; MTSSL labeling.
Bottom row. Left: iso-cytochrome c: folding; MTSSL labeling; wide distance ranges. ***Center***: Ribonucleotide reductase (yeast), RNR: using radical cofactors and substrate; unlike spin labels. ***Right***: Gramicidin doubly-labeled at C,N-termini: membrane-associated peptide; iod-acetamide labeling; equilibrium of different forms; aggregation aspects. ***Far right***: Linear polyproline peptide Ac-OO-TOAC-PPPPPPP-TOAC-OO-Amide.
The peptide model was built with Insight II and edited using Pymol. The measured distance 27.86 Å is between nitrogen atoms of NO moieties of two TOAC residues. DQC provided 26.8 Å distance, 1.5 Å FWHM distribution. (Borbat & Kallenbach, unpublished data).

1. Introduction

Continuous-wave (cw) and pulsed ESR have been extensively applied to biological problems in the context of molecular dynamics [1–4] and are now increasingly applied to study biomolecular structure and function. cw ESR has been used to measure distances in the range of 6–20 Å between pairs of nitroxide spin labels [5–14]. Distance measurements using pulse ESR methods, a major advance in this area, are currently able to deliver long-distance constraints in the range of 10–80 Å [15–37]. The distance constraints from pulse ESR can for example be used to establish protein folding or orient and dock proteins and their subunits, yielding useful insights into the structure of a protein or a protein complex. They can also aid in refinement of NMR data. We refer to this emerging methodology as 'pulsed dipolar (ESR) spectroscopy' or PDS for short.

The PDS method[1] of double electron-electron resonance (DEER, also known as PELDOR) [38–41], was introduced more than two decades ago to circumvent problems in isolating weak electron-electron dipolar couplings from electron-spin-echo decays, which are usually dominated by relaxation and nuclear modulation effects [42, 43]. But there were few applications until the development of site-directed spin labeling (SDSL) as a useful tool of structural biology [44–46], as well as a modified version of DEER enabling its commercial implementation, and last but not least major efforts of dissemination. During that time, other pulsed methods of distance measurements were introduced [47–52], with the most useful being double-quantum coherence, (DQC) ESR [47, 48] (or DQC for short). Applications of DEER and DQC, to structural problems in biology have rapidly grown in number and scope in the last few years [3, 16, 18, 22, 23, 25, 28, 32, 33, 36, 37, 49, 53–56], with several reviews outlining the distance measurements [56–63].

We illustrate in this commentary PDS applications and methodology (both DQC and DEER) through examples from our laboratory, which cover many aspects of its applications to biomolecular structure and function. We only present here a short synopsis.

2. Distance Measurements

As ESR spectroscopists know well, the dipole-dipole part of the spin-Hamiltonian, H_{dd} between electron spins 1 and 2, (as relevant within this context) is given by

$$\frac{H_{dd}}{\hbar} = \frac{\gamma_e^2\hbar}{r^3}(3\cos^2\theta - 1) \times\left[S_{1z}S_{2z} - \frac{1}{4}(S_1^+S_2^- + S_1^-S_2^+)\right] \quad (1)$$

in high magnetic fields, where the non-secular terms (not shown) are unimportant [64]. One usually uses the point dipole approximation in employing Eq. (1), i.e. the electron spins are far enough apart that their distributions (in e.g. nitroxide p-π orbitals) are unimportant, (i.e. r > 5 Å for nitroxides). In Eq. (1), θ is the angle between the direction of the static magnetic field B_0 and **r** = (r, θ). The term in $S_{1z}S_{2z}$ in Eq. (1) is known as the secular term, and that in $S_1^{\pm}S_2^{\mp}$ the pseudosecular term. The dipolar coupling in frequency units may be written as

$$A(r,\theta) = \omega_d(1 - 3\cos^2\theta) \quad (2)$$

with

$$\omega_d = \gamma_e^2\hbar/r^3. \quad (3)$$

It leads to a splitting of the resonant line of each spin into a doublet[2]. For the case of unlike spins, i.e. $\omega_d << |\omega_1 - \omega_2|$, (where ω_1 and ω_2 are the resonant frequencies of the two electron spins in the absence of dipolar coupling) the splitting is by $|A|$; the precise value of A depends on the angle θ, yielding a range of values of A from $-2\omega_d$ to $+\omega_d$. The PDS dipolar spectrum provides this splitting, which is shown in Fig. 1 as a function of the angle θ, obtained from a macroscopically aligned frozen sample. In the usual case of an isotropic frozen sample, one observes an average over θ, which yields a distinct dipolar spectrum, known as a Pake doublet [65], (cf. Fig. 2a). It shows a prominent splitting of ω_d, corresponding to θ = 90°, and another splitting of $2\omega_d$, corresponding to θ = 0°. The distance r is immediately and accurately obtained from a measurement of ω_d. This more familiar case of unlike spins corresponds to considering only the secular term in Eq. (1) and ignoring the pseudosecular term. In the case of like spins, i.e. $\omega_d >> |\omega_1 - \omega_2|$, then the pseudosecular terms become important (a fact less appreciated) and Eq. (3) becomes

$$\omega_d = 3\gamma_e^2\hbar/2r^3.$$

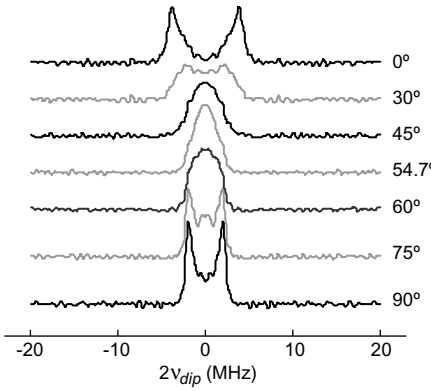

Fig. 1. An experimental dipolar spectrum of spin-labeled gramicidin A [24] obtained by 4-pulse DEER at several orientations in a macroscopically aligned lipid membrane bilayer of DMPC.

[1] Both acronyms, PELDOR and DEER do not indicate the fact that they are solely concerned with dipolar couplings rather than dynamics. Also DQC obscures its application to dipolar couplings. Thus we prefer to use PDS to make more explicit the function of the methods.

[2] We leave out a discussion of electronic exchange, which for nitroxides is not significant above about 10 Å.

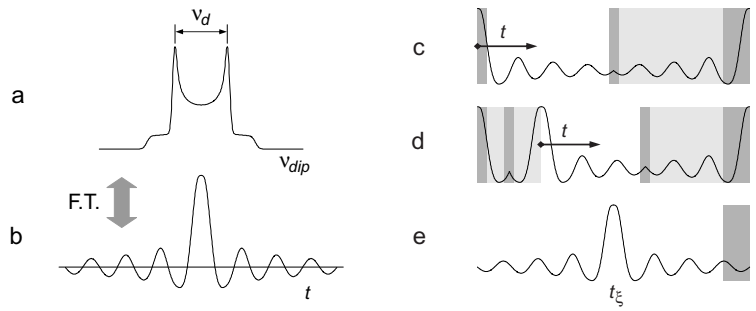

Fig. 2. a, **b** A dipolar spectrum in isotropic media (Pake doublet) from cosine Fourier transformation of the simulated time-domain signal. **c**, **d** and **e** Forms of dipolar signals for 3-pulse DEER (PELDOR), 4-pulse DEER, and DQC respectively. Shaded areas indicate repeats of the signals, which are not sampled. Wide dark bars depict dead-time zones, i.e. representing true dead-time when no signal can be detected due to overload conditions. Narrow bars correspond to conditions when the signal may be corrupted due to overlapping of pulses in the amplifier or due to bichromatic irradiation and step-wise signal phase shifts when pulses change their order. It is clear that 4-pulse DEER and DQC provide dead-time-free signals, with no off-resonance excitation effects in the latter. Usually, only one half of the signal is recorded in all three methods.

Otherwise the results (cf. Fig. 2) are equivalent. The intermediate case of $\omega_d \approx |\omega_1 - \omega_2|$ is more complex, and is handled by careful simulation using Eq. (1) including both secular and pseudosecular terms, (and using the full spin-Hamiltonian). In the case of nitroxide spin labels, the two nitroxide spins in a given molecule usually have their ω_1 and ω_2 substantially different. This arises from their different orientations with respect to the B_0 field, so their effective hf and *g* values (arising from their hf and *g* tensors) are different. At typical ESR frequencies this means that the unlike spin limit is valid generally only for ≥20 Å (9–17 GHz ESR).

If ω_d is sufficiently large, it can be determined from the broadening of the nitroxide cw ESR spectrum [7] but this is likely to fall into the regime where pseudo-secular terms are significant. Smaller couplings, ω_d require using pulse ESR methods. In all cases, accurate values of distances are produced from the measured dipolar couplings.

3. How do ESR and PDS Compare to Other Methods?

a) ESR vs. X-Rays and NMR

The primary sources of structure at atomic resolution are, of course, X-ray crystallography and NMR. Many biomolecules, however, are not amenable to study by NMR or crystallography for reasons such as insufficient quantities, inability to grow diffraction quality crystals, large molecular weight, poor solubility, or lack of stability, etc. Currently, determining the structure of a relatively small membrane protein is a challenge for both NMR and crystallography. The notable virtues of ESR-based limited structural methods compared to X-ray and NMR methods are that the former require only tiny amounts (nano- to picomole [66] of proteins or other biomolecules), and they can be studied in a variety of environments, e.g. dilute solutions, micelles, lipid vesicles, native membranes, supported lipid bilayers, and more. There is no need to grow crystals or be concerned with long-term protein stability at high concentrations. Large biomolecules or complexes that are beyond the range of NMR or X-ray methods are not a major limitation; even unstable or transient biomolecules can be captured and studied. It is worth mentioning that PDS often relies on the availability of partial structural information provided by X-rays or NMR; and it may be employed synergistically, as was the case in recent applications [18, 32, 36].

b) ESR vs. FRET

FRET also provides distances over a range comparable to ESR. Its very high sensitivity, access to longer distances, and ability to operate at biological temperatures makes it a potent tool, but PDS has its distinct virtues. It has now become routine to express, purify, and spin-label dozens of mutants for nitroxide scan [67–69] or to produce and label a set of cysteine double-mutants for distance measurements. The distance between nitroxides as well as distance distributions is more accurately determined than between chromophores, since it is directly obtained from a simple frequency measurement, and there are no uncertainties in κ^2 as in FRET. There is usually a single type of reporter group, which is often a methanethiosulfonate spin label (MTSSL), and in most cases it introduces only a small perturbation to the protein structure and function. Since the nitroxide side-chains are smaller in size than most fluorescent labels, the uncertainty of their positions relative to the backbone is less. A drawback of PDS, as well as of FRET, is that a limited number of constraints, which are themselves the distances between the reporter groups rather than the backbone C_α carbons may only provide limited insights into the structure. However, the detailed 3D structure is not always required, e.g. to elucidate the functional mechanism. But the fact that the distances are measured between the reporter groups does lead to a challenge in translating them into distances between the C_α carbons at the labeled sites. Modeling efforts, to overcome this, are in early stages of development [70].

c) CW and pulse ESR

CW ESR has been most often applied to nitroxides, whose powder spectra are dominated by the inhomogeneous broadenings from nitrogen hyperfine (hf) and *g*-tensors, and unresolved proton hf couplings. One has to extract what usually is a small broadening effect introduced by the dipole-dipole interactions between the spin labels to the nitroxide powder spectra. This is usually accomplished by spectral deconvolution [12] or by a rigorous spectral simulation with a multiple-parameter fit [7]. This often requires the spectra from singly-labeled species as a reference for the background broadening, which is a complication and not always an option. Incomplete spin labeling makes the task more complex [71]. For distances less than 15 Å, the dipolar coupling approaches other inhomogeneous spectral broadenings and then can be more easily inferred from cw ESR spectra. CW ESR is thus practical for short distances up to a maximum of ca. 15–20 Å, with the values for distances under 15 Å being more reliable [71].

Pulsed ESR is based on detecting a spin-echo, wherein the inhomogeneous spectral broadening cancels. Spin echo temporal evolution is governed by the weaker effects of spin relaxation and not refocused electron-electron dipolar and exchange couplings, and electron-nuclear super-hyperfine and nuclear quadrupole couplings. The dipolar and exchange coupling can be isolated from the rest by means of a suitable pulse sequence. This also helps to alleviate the problem caused by the presence of single labeled molecules. The direct signal from them is filtered out in some forms of PDS, but they do contribute to the background intermolecular dipolar signal, ▶

which is best suppressed by working at low concentrations. PDS is routinely used for distances longer than 15–20 Å [15, 18, 19, 22, 59, 32], and it works well all the way down to 10 Å [25], thus significantly overlapping with the cw ESR range, but it is much less affected by inefficient labeling and can readily yield distance distributions. The sensitivity of PDS is rather high as we show.

4. PDS at Work

The development of PDS has involved two stages. In the first, the fundamental aspects and details of the methods had to be developed, so they could be applied in the context of biomolecular structure and related applications. The second stage has been its practical use, wherein subtle details are of lesser concern, with the main goal being to solve structures by distance constraints. Substantial progress has been made, rendering such applications routine [18, 22, 32, 33, 60, 62, 63], although there is much room for further developments.

To illustrate the current stage of development of PDS at ACERT, we have assembled a small zoo, populated with selected species of proteins and peptides that we have studied, which are portrayed on the Front Cover. Although we cannot show here most of the signals and distance distributions, we note that they are of a very good or excellent quality, well in line with ACERT standards. The collage on the front cover represents cases that illustrate the following aspects: i) Protein environment (soluble, reconstituted in detergent micelles or liposomes, in natural membrane environments); ii) Oligomerization status (establishing the state of oligomerization, circumventing problems of multiple spins, heterodimers, tandem dimers and tetramers); iii) The state of folding (unfolding/refolding equilibrium in denaturants and freeze-trapped); iv) Spin-labeling aspects (naturally-occurring radicals, spin-labeled substrates and inhibitors, MTSSL labeling, other nitroxide labels, termini labeling); v) PDS modes (single distance, multiple distances, triangulation); vi) PDS methods used (DQC, DEER; vii) Peptides (water soluble, organic solvents, lipid vesicles, macroscopically-aligned lipids; dimers, conformers, aggregates, spin-counting, equilibrium, affinity, membrane composition); viii) Functional studies (capturing functional states with substrate mimetics, pH, ligands); ix) Data processing aspects (background removal, distance distribution and refinement, distance embedding, rigid-body modeling); x) Oligonucleotides (long-distance constraints to aid NMR); xi) Protein complexes (binding and docking, tertiary and ternary structure, large supamolecular complexes, mobile subunits and domains).

a) Single-distance measurement

When a rough structure or the oligomeric state of a protein complex is of interest, a few distances may suffice [15]. This mode of PDS has been used most often to produce a critical distance or its change, providing insights into a key structural aspect, such as the location of a binding interface or the extent of conformational change.

b) Multiple-distance measurements

Obtaining more detailed structural information is usually more involved, since it requires obtaining several distances in order to select among possible conformations of a protein or a protein complex, by checking that all experimental distances are consistent with the model [35, 53]. The sites should be accessible for the spin-labeling reagent, and they should not alter protein structure or function; this may limit their selection.

c) Triangulation

The 'triangulation' approach to protein mapping [20, 32], is based on obtaining a network of distance constraints from a set of spin labeled sites such that they uniquely define the coordinates of all (or most) of the sites. A sufficiently large rigid distance network (scaffold) based on tetrahedra [20, 32] strongly restrains positions of spin labels and thereby the possible conformations of the protein (cf. Fig. 3). Such constraints can be used to solve the protein structure at a low resolution of ±5 Å. This task can be accomplished by making a sufficient number of double mutations and then measuring the distances between the respective pairs of spin labels in a 'one-at-a-time' manner. It is not feasible, in general, to obtain distances simultaneously amongst several spin labels due to the flexibility of the side-chains and the structural heterogeneity of proteins, which usually yield fairly broad distributions in each distance. However, there can be favorable cases [16, 32, 72] (cf. Fig. 4).

d) Oligomeric proteins

Many proteins are oligomeric and they require additional care to obtain the required set of constraints. Even the simplest case of a dimeric protein of CheA [32] required one to select mutation sites such that the measured distance could be isolated from other distances possible between more than two labels. Doubly-labeled homodimeric protein carries four spin labels, therefore six distances are possible in general. They can be resolved in cases when all (or most of them) are strongly immobilized. Otherwise the distance of interest should be well-isolated from the rest. This approach was successfully implemented in triangulation study of CheW binding to CheA [32, 62]. In the case of tetrameric membrane channels (KcsA), tandem dimers were also used to provide better resolved dipolar spectra. Tandem tetramers can also be expressed and folded for KcsA or KvAP, and they can be applied to set up triangulation. Another aspect of work with oligomeric proteins is to establish their oligomeric state in native environment which was accomplished for monoamineoxidase (MAO) in the outer mitrochondrial membrane [37].

e) Protein complex

The potential problems are structural heterogeneity of the complex, and low affinity leading to weak dipolar signals compared to that from single-labeled proteins. This task is better suited for DQC, conducted at low concentrations and at a high frequency, possibly in Ka- or W-band, which may enable detection of just a few percent of dimers in a pool of single-labeled protomers.

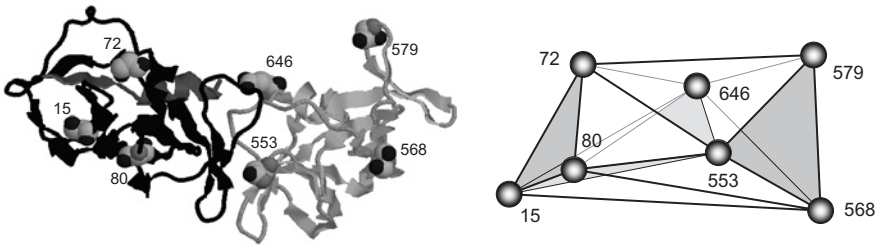

Fig. 3. Structure of P4/P5/CheW complex (P4 is not shown) determined by PDS and confirmed by X-ray. [32]. Residues mutated to nitroxides for PDS are shown in a space-fill representation; (right) also shows the rigid triangulation grid based on tetrahedra.

f) Embedding PDS constraints and rigid-body modeling

In the case of tertiary or ternary structure, which includes aspects such as the relative position of protein subunits or protein docking, knowing all possible distances amongst several labeling sites makes it possible to transform them into molecular coordinates of the sites by means of a generic method of embedding based on metric matrix distance geometry. This task was accomplished for the CheA/CheW complex; the results are shown in Fig. 3. When some constraints are missing, the problem of embedding requires a more advanced method, e.g. based on CNS software [73], which is up to the task. This approach was successfully applied to dock CheW to its binding site in CheA and also to determine the tertiary structure of α-Synuclein [63]. A recent proposal suggests using spin-label rotamer libraries [74].

g) Difficult labeling cases

Not all proteins can be successfully labeled with nitroxide using the SDSL approach. Some cysteines can be functional (RNR, MAO), or the protein may be destabilized, or its function significantly altered. In this case there are currently few approaches. It is possible to measure distances between radical cofactors to establish oligomerization state, quantify major structural change, or the pathway for electron transfer in some cases. Radical cofactors and spin-labeled inhibitors were used in the case of MAO [37], depending on protein environments, with only a spin-labeled inhibitor being suitable for labeling in native membranes. Optimistically, one could rely on future developments such as unnatural amino-acid mutogenesis that may permit spin-labeling with high specificity, spin label incorporation via protein splicing technology, etc. The low amounts required for PDS may be well in-line with these trends.

h) Structural and conformational heterogeneity, protein folding

Iso-cytochrome C unfolded by varying concentration of denaturants was explored at the outset of the L-curve Tikhonov and MEM method development with the goal of exploring the utility of distance distributions from PDS to study kinetically trapped folding intermediates [72, 91]. α-Synuclein, (αS), is unstructured in solution, but it assumes helical structures on micelle or membrane surfaces. The tertiary structure of αS was based on the multiple-distance approach and rigid-body modeling [63], and they have enabled us to establish the arrangement of the two helical subunits.

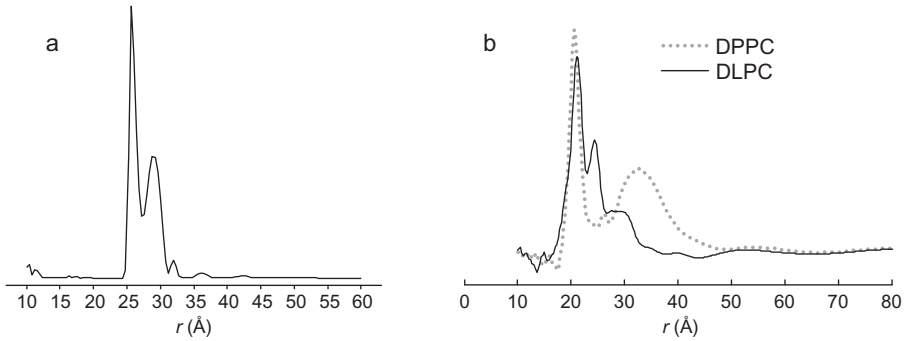

Fig. 4. a MEM reconstruction of distances between two symmetry-related sites in the dimerization interface of histidine kinase, CheA from *T. maritima* spin-labeled at site 318. The data were obtained using DQC at 17.4 GHz. Two peaks in P(r) separated by 2.5 Å probably indicate the presence of two distinct conformations in this part of the protein. **b** Distance distribution reconstructed by the L-curve Tikhonov regularization method applied to 17.4 GHz DEER data from a doubly-labeled gramicidin A diluted with unlabeled gramicidin A in DLPC (solid line) and DPPC (dashed line). Gramicidin A is a monomer in DLPC with the distance between labels of about 20 Å, but also forms some double helical dimers in DPPC (solid line) visible as a broad distribution at 34 Å.

All the above examples and applications have been addressed mainly by using just two PDS methods, which have worked the best, namely DQC and DEER. Taken together they cover most practical aspects that can arise in structure determination by ESR. Both have their strengths and weaknesses, which tend not to overlap.

5. PDS Toolbox

a) 3-pulse DEER

DEER in its original 3-pulse form [40] is based on the two-pulse primary spin-echo π/2-τ-π–τ–echo sequence to which a 3rd pumping π-pulse is added. The primary echo from the π/2- and π-pulses, separated by time interval τ, is applied to spins resonating at the frequency ω_A, to form an echo at the time 2τ after the π/2-pulse. These spins are commonly referred to as A spins. The third (pumping) pulse is applied at the resonant frequency ω_A (at a variable time t) sufficiently different from ω_B that it does not have any direct effect on the A spins but instead inverts the spins resonating at ω_B, i.e. the B spins. The B spins, at a distance r from the A spins yield the electron dipolar coupling A (cf. Eq. (2)), which splits the resonant line at ω_A into a doublet. Thus flipping a B spin inverts the sign of the coupling sensed by the A spin. This results in the instant shift of the Larmor precession frequency of spins A; it was shown in [40] that the effect manifests itself as a modulation of the spin-echo amplitude, V(t), which for like spins is:

$$V(t) = V_0[1 - p(1 - \cos A(r,\theta)t)] \quad \text{for } 0 < t < \tau. \tag{4}$$

Here V_0 is the echo amplitude in the absence of the pumping pulse and p is the probability of flipping spin B. Powder averaging of $V(t)$ over an isotropic distribution of orientations of **r**, under the simplifying assumption of random orientation of the magnetic tensors of the A and B spins relative to r produces a decaying oscillatory signal (cf. Fig. 2c):

$$V(t) = V_0[1 - p(1 - u(\omega_d t))], \tag{5}$$

where

$$u(\omega_d t) = \int_0^{\pi/2} \cos[\omega_d(1 - 3\cos^2\theta)t]\,d(\cos\theta) \tag{6}$$

is the desired ('dipolar') signal, oscillating with the frequency of $\nu_d = \omega_d/2\pi$, from which r is calculated as r[Å] = 10(52.04/ν_d[MHz])$^{1/3}$. Cosine Fourier transformation of $u(\omega_d t)$ vs. $2t$ (that is the full dipolar evolution time) yields the dipolar spectrum with the shape of a Pake doublet (cf. Fig. 2a). The remaining (and the larger) part of $V(t)$ amounts to background, which makes it difficult and sometimes impossible to separate weak $u(\omega_d t)$ from the effects modifying and destabilizing the background, which constitutes the major source of errors.

Equations (4) and (5) thus should be considered as a reasonable approximation for DEER, which is suitable for the majority of cases encountered in biological applications of PDS. In reality, a number of factors affect the signal, and their effects usually cannot be written in closed form or are unwieldy [48, 75–77]. What is significant is that DEER achieves a good separation of the dipolar coupling from relaxation effects in most practical cases, because the time between the π/2 and π spin-echo pulses at ω_A is constant, (i.e. τ in Fig. 5 is constant in the experiment; this is referred to as a constant time pulse se-

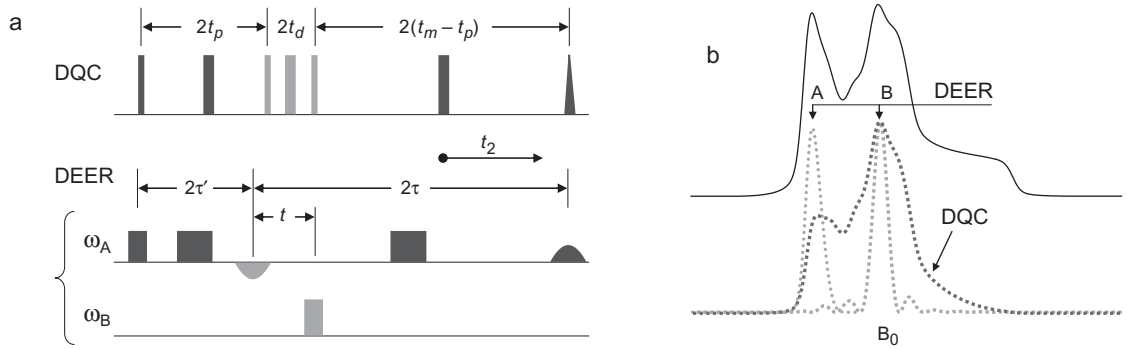

Fig. 5. a 6-pulse DQC (top) and 4-pulse DEER (bottom) sequences: The DQC 6-pulse sequence [47, 48] is based on intense pulses in order to probe the dipolar coupling between (nearly) all intramolecular pairs of nitroxide spins. The reference point $t_\xi = 0$ is well-defined due to the very short pulses used in DQC. The 4-pulse form of DEER is based on softer selective pulses, with detection of the refocused primary echo formed at ω_A of A-spins. **b** Excitation of the nitroxide spectrum at 17.3 GHz for DQC and DEER. The ^{14}N nitroxide ESR spectrum is plotted as a solid line and the spectral excitation profiles are plotted as dashed lines. The detection frequency in DEER is set at the low field edge of the spectrum (A) and the pump pulse frequency corresponds to positioning it at the center (B). The pumping pulse is 4 G (45 ns π-pulse) in DEER; The wide DQC excitation profile corresponds to a 48 G (3.7 ns) π-pulse.

quence), and relaxation effects introduced by the pumping pulse can normally be ignored. Nuclear ESEEM is also considerably suppressed, but still could be an issue.

b) The newer and better methods

i. 4-pulse DEER

The more recent methods of 4-pulse DEER [78] and 6-pulse DQC [1, 4, 20, 47, 48] are illustrated in Fig. 5. The 4-pulse DEER sequence is an improvement over 3-pulse DEER. It is based on the 3-pulse spin-echo sequence π/2-τ′-π-(τ+τ′)-π-τ-echo, which refocuses the primary echo formed by the first two pulses. The additional pumping pulse at ω_B is varied in time, t between the π-pulses at ω_A (cf. Fig. 5). Both τ and τ′ are fixed, thus relaxation does not modify the signal envelope recorded vs. position of the pumping pulse. The signal is described by Eqs. (4) and (5) at the same level of approximation as 3-pulse DEER (also cf. Fig. 2d). This pulse sequence substantially simplifies its technical implementation, since the starting point ($t = 0$) is shifted away from the second pulse by τ′. This has enabled convenient commercial implementation.

ii. 6-pulse DQC

The 6-pulse DQC pulse sequence π/2-t_p-π-t_p-π/2-t_d-π-t_d-π/2-(t_m-t_p)-π-(t_m-t_p)-echo (cf. Fig. 5) is based on a different principle. All pulses are applied at the same frequency ω_A, and it is important that they all be intense in order to excite the whole spectral distribution of spins, i.e. all the spins are regarded as A spins. The first interval, $2t_p$ is used to let the normal single-quantum coherence with spin character $S_{1y} + S_{2y}$ evolve into what is known as anti-phase single-quantum coherence between the coupled spins with spin character $S_{1x}S_{2z} + S_{2x}S_{1z}$. Then the π/2-$t_d$-π-$t_d$-π/2-pulse 'sandwich' (hatched bars in Fig. 5) converts this coherence into double-quantum coherence with spin character $S_{1x}S_{2y} + S_{1y}S_{2x}$ (by means of the first π/2-pulse), then refocuses it by means of the π-pulse, only to convert it back to (unobservable) anti-phase coherence (by means of the last π/2-pulse), which evolves back into the observable coherence $S_{1y} + S_{2y}$, giving rise to the echo. Both spins participate equally in the process. The first and the last π-pulses of the 6-pulse sequence are used to refocus in-phase and anti-phase coherences, thereby respectively enhancing the effectiveness of the double-quantum filtering (DQF) 'sandwich', and producing the echo at time $2t_m + 2t_d$. The signal in the ideal limiting case of intense and non-selective pulses can be written as [47, 48]

$$V = -V_0 \sin A(r,\theta)t_p \sin[A(r,\theta)(t_m - t_p)]$$
$$= \frac{V_0}{2}[\cos A(r,\theta)t_m - \cos A(r,\theta)t_\xi]\,. \qquad (7)$$

The signal is recorded vs. $t_\xi = t_m - 2t_p$, with t_m kept constant in order to keep relaxation effects, (which decay exponentially in time) constant. (Also t_d is kept short and constant.) Powder averaging gives

$$V = \frac{V_0}{2}[u(\omega_d, t_m) - u(\omega_d, t_\xi)] \qquad (8)$$

with $u(\omega_d, t_\xi)$ is given by Eq. (6). For large $\omega_d t_m$ the first term in Eq. (7), which is constant in t_ξ, is close to zero, leaving just the desired 'dipolar' signal. The important feature of the double quantum coherence sandwich is that it very effectively filters out the single quantum signals arising from the individual spins, and only passes the signal from the interacting part of the two spins, which just contain the dipolar oscillations. The only background that can develop is from the double quantum coherence signal that originates from the bath of surrounding spins, i.e. from intermolecular electron-electron dipolar interactions with other doubly-labeled molecules, (and singly-labeled molecules when they are present). The signal envelope $V(t_\xi)$ is symmetric with respect to $t_\xi = 0$. This is referred to as being dead-time free, since the dipolar oscillations are a maximum at $t_\xi = 0$ (cf. cosine term in Eq. (7)).

Relaxation effects that decay exponentially but non-linearly in time in the exponent [20], or substantial differences in T_2's from the two spins, can modify the signal as the positions of refocusing pulses and DQF are not fixed. The 6-pulse sequence generates a number of echoes, but with the proper phase cycling only the dipolar modulation of the double-quantum filtered echo is detected. The details can be found in [48].

The DQC experiment maintains phase coherence between the two coupled spins and treats them equally, whereas in DEER, phase coherence between the two coupled spins is of no importance. The independence of tuning of the pulse conditions at both frequencies, as well as its applicability to widely separated spectra, makes the DEER sequence quite flexible. Nevertheless, it can be shown that the dipolar signal recorded in DEER is based on the same type of evolution of in-phase and anti-phase coherences as in DQC. This is also the case with other related pulse sequences [48]. Although it may look complex, the DQC experiment, once it is set up using adequate equipment, is rather simple to use. The similarity in DQC and DEER means that the maximum useful time of ▶

the experiment (i.e. $2t_m$) in DQC and 2τ in DEER will be comparable, except for respective differences in signal-to-noise (SNR) as discussed below.

DQC and DEER, even though both are not perfect, have proven to be the most useful methods, and together they address a wide range of applications. Additional methods of occasional use were introduced elsewhere [49–52, 79].

6. Relaxation and Distance Range

The amplitude of the primary echo V_0 decays with pulse separation due to phase relaxation. Therefore the maximum dipolar evolution time interval, t_{max} available for recording $V(t)$ is ultimately limited by the phase memory time, T_m. In the simplest case, $V(t) = V_0\exp(-2t/T_m)$. This limits the maximum distance, r_{max} that one can measure, over a reasonable period of signal averaging. Depending on the signal strength, t_{max} is ca. 1–3 T_m and cannot be extended much further. Here t_{max} is essentially $2t_m$ in DQC and $2(\tau' + \tau)$ in DEER (cf. Fig. 5). The largest measurable distance, r_{max} is proportional to $t_{max}^{1/3}$ in order to recover the dipolar oscillation [48]. Thus only a minor increase in r_{max} can be made by increasing t_{max}, and this would necessarily require a large increase in signal averaging. For nitroxide-labeled proteins, T_m is largely determined by the dynamics of the nearby protons [80–82], especially those from methyl groups, leading to the simple exponential decay expressed above with T_m in the range of 1–2 μs for buried or partially buried labels. Such relaxation times are typical for hydrophobic environments that are encountered in lipid membranes and the protein interior [81]. This permits an r_{max} of typically 50 Å [134]. For water-exposed labels, relaxation at longer τ is dominated by $\exp[-(2\tau/T_m)^\kappa]$ with $\kappa \sim 1.5$–2.5 and $T_m \sim 3$–4 μs [81]. A quadratic term in the exponent is governed by the nuclear spin diffusion mechanism [83, 84]. This permits an r_{max} of typically ~55–60 Å (or ~70–75 Å with low accuracy). Such types of relaxation could be partially suppressed by multiple refocusing and/or using deuterated solvent [19, 48, 85, 86]. This could extend t_{max} to ca. 6–8 μs in favorable cases [80], i.e. much less than in D2O/glycerol-d8, as there still is a bath of protons of the protein itself [80]. Using 6-pulse DQC helps to extend t_{max} when T_m is dominated by nuclear spin diffusion [19, 48]. This permits a more accurate estimate of r_{max} to ca. 70 Å. Further improvement would require much greater effort such as partial or complete protein deuteration, and this might extend r_{max} to 100–130 Å and make distances up to 80 Å much more accurate.

The longitudinal relaxation time, T_1, determines how frequently the pulse sequence can be repeated, (usually no more frequently than $1.5/T_1$), and consequently the rate at which the data can be averaged. Both T_1 and T_2 are temperature dependent, as is the signal amplitude, which depends on the Boltzmann factor for spins in the dc magnetic field. The combined effect of all these aspects is such that for proteins in water solution or in membranes the optimal temperature as a rule is in the range of 50–70 K for both DQC and DEER.

We summarize next the limiting distance ranges and what is optimum.

a) Long distances

As noted above, the ability to measure very long distances is limited by the phase memory time, T_m and for proteins 65–75 Å is about the upper limit with current technology. Also, distances measured in this range are typically not very accurate. This situation could be radically improved by protein deuteration. Alternatively, with a good spin labeling strategy, such long distances may be avoided.

b) Short distances

The π-pulse excites a spectral extent (in Gauss) of about B_1. It is necessary to excite both components of the Pake doublet in DEER, which normally uses π-pulses longer than 20 ns (B_1 of ~9 G). This provides a lower limit to DEER of ca. 15–20 Å (cf. Fig. 6). However, π-pulses of 30–60 ns width are typical, since they provide a cleaner implementation of the method, which requires that the pump pulse and observing pulses do not overlap in spectral extent. This tends to limit DEER to ca. 20 Å. The sensitivity to shorter distances decreases significantly because the coupling increases and both components of the Pake doublet can no longer be adequately excited [87]. Also, account must be taken of strong dipolar coupling during these long pulses [75]. (We also note that longer pulses render ESEEM effects negligible because of sufficient spectral separation.)

DQC uses intense pulses with B_1 of 30 G or greater, hence it can access distances as short as ca. 10 Å [25](cf. Figs. 6 and 7). In this case the pseudosecular part of the dipolar term in the spin-Hamiltonian (cf. Eq. (1)) cannot be neglected, but this can be accounted for in rigorous numerical simulations [48]. The short distance range is more appropriate however for organic biradicals, buried spin labels or radical cofactors, TOAC, and similar cases, when radicals are substantially immobilized and their geometry is known or can be deduced. This range is less desirable for typical nitroxide labels with long tethers, with uncertain geometry.

c) Optimal range of distances

In our experience an optimal range of distances for the purposes of PDS is within 20–50 Å (45 Å for membrane proteins, whose T_m's are 0.7–1 μs), even though larger distances can be measured with a longer period of signal averaging, but usually with reduced accuracy. Distances shorter than 20 Å introduce a relatively larger uncertainty ▶

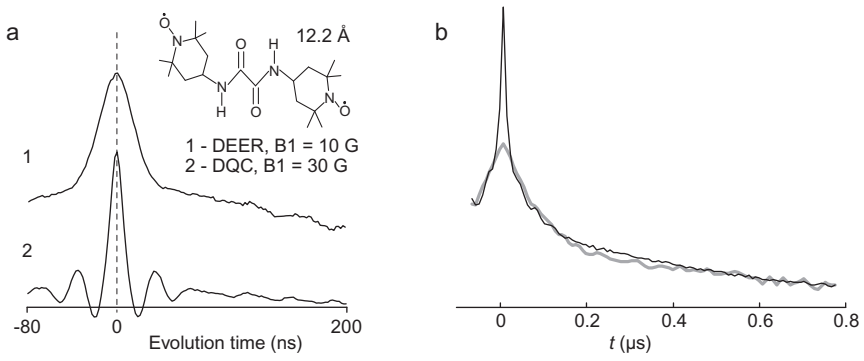

Fig. 6. a The challenges of short distances. DQC and DEER were applied to a rigid 12.2 Å nitroxide biradical. Detection pulses in DEER were 16/32/32 ns, the pumping pulse was 18 ns (B_1 ~ 10 G). This is found to be insufficient to properly excite the dipolar spectrum. DQC using 6.2 ns π-pulse (B_1 ~ 30 G) develops the ~30 MHz oscillations very cleanly. The longer pulses of DEER lead to a spread in the refocusing point of different spin packets, and the weaker B_1, both smear out the high-frequency dipolar oscillations. (The biradical courtesy of R. G. Griffin). **b** The superimposed DQC (solid line) and DEER (dotted line) signals obtained for the CheAΔ289 heterodimer doubly-labeled in one of two protomers at two close sites [32]. DQC is able to detect a broader range of distances.

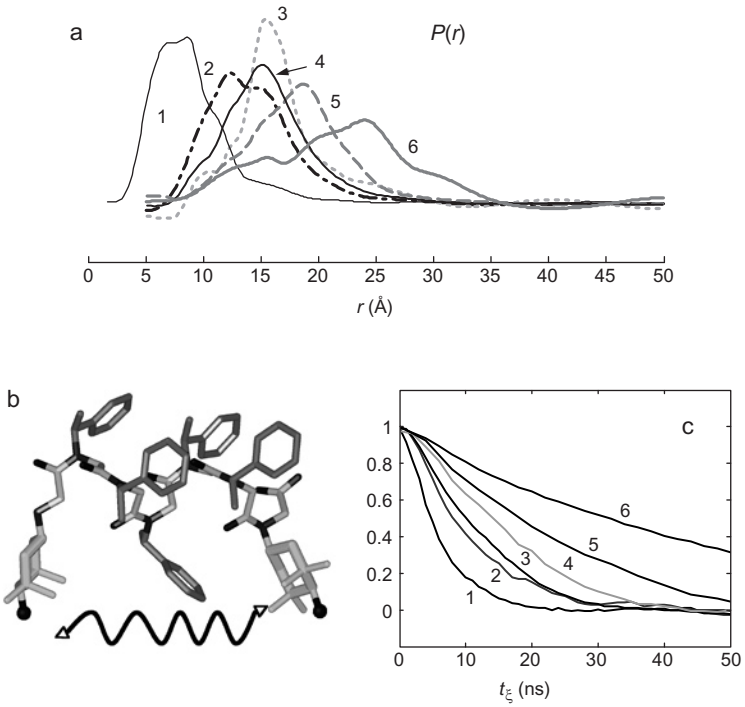

Fig. 7. a Distance distributions for a set of doubly-labeled peptoids (**b**) with a range of end-to-end distances [25]. **c** Shows time-domain signals, which for the shortest distance, decay is less than 10 ns.

in estimating the C_α-C_α distances. Measurement of distances in the optimal range is fast and accurate in most cases. The labeling sites and distance network should thus be chosen such that they provide optimal conditions for PDS, by increasing the relative number of optimal distances, as needed. Optimal conditions are not readily available for oligomeric proteins due to multiple labels, and their typically large size. For an unknown structure, a preliminary scanning by several trial measurements may be very helpful.

7. Sensitivity of PDS

The sensitivity of PDS techniques, specifically DQC and DEER, has been discussed in [48], where the main criterion for sensitivity was based on the ability to perform a successful experiment, (i.e. of reliably measuring a distance) in a reasonable period of time. It was chosen to correspond to an acceptable SNR, nominally taken as a S_{acc} of 10, which has to be attained in an acceptable time of experiment nominally taken as 8 hrs of signal averaging. Such a SNR would make it possible to obtain a desired distance, given a sufficient length of, t_{max}. However, a S_{acc} of 10 is a bare minimum, and we usually require a SNR of at least 50 [72].

Even though it is possible to estimate sensitivity rather accurately from first principles [88], we prefer to use an experimental calibration in the spirit of [48] based on measurement of the spin echo amplitude using a two-pulse primary echo (PE). Such an experiment provides the SNR for a single-shot, S_1(PE).

The calibration of DQC and DEER has been conducted for our pulse ESR spectrometer [32, 89] at the working frequency of 17.35 GHz on a nitroxide sample of 4-hydroxy TEMPO in a vitrified solution of 50% (w/v) glycerol in H_2O with a 20 μM spin concentration in a 10 μL sample volume at 70 K, where most PDS measurements are performed. The DEER calibration used a primary echo [90] generated by π/2-π pulses (π-pulse of 32 ns) separated by 80 ns, with the pulses applied at the low-field edge of the nitroxide spectrum. A similar DQC calibration was based on π/2-π pulses with a 6 ns π-pulse, and the same separation as in DEER, but pulses were applied in the middle of the spectrum. For the two measurements, the ratio of the echo amplitudes relevant for (DQC vs. DEER) was ca. 6.5 and the ratio of SNR's of the single-shot signals at the condition of optimal signal reception (i.e. given by the integration of the spin echo in the time window defined by the time points corresponding to 0.7 of the echo amplitude) was ca. 3.0, i.e. $S_1 \approx 0.42$ μM^{-1} (DEER) and $S_1 \approx 1.25$ μM^{-1} (DQC).

Based on these numbers, the estimates of the dipolar signals for the two methods according to the analyses given in [47, 48] are summarized as follows. For 4-pulse DEER with 16/32/32 ns pulses in the detection mode and a 32 ns pump pulse, S_1 is 0.084 μM^{-1}, and for DQC based on a 3/6/3/6/3/6 ns pulse sequence, S_1 is 0.3 μM^{-1}, i.e. it is greater for DQC by a factor of 3.6. This ratio is supported by our experimental observations, (e.g. Fig. 8). Using the sensitivity analysis of [48] we estimate the SNR of the raw data of the full PDS experiment as

$$SNR = 2S_1 x^2 C\eta_c K(f,T_1)(ft_{exp}/n)^{1/2} \times \exp\left(-\frac{2t_{max}}{T_m} - 2kxCGt_{max}\right). \quad (9)$$

Here, t_{exp} is the duration of the experimental data acquisition; f is the pulse sequence repetition frequency; n is the number of data points in the record[3]; C is the doubly-labeled protein concentration (μM); η_c is the ratio of the sample volume (≤15 μL) to that used in the calibration (i.e. 10 μL). The terms in the exponent are consistent with those given in [48], namely the first accounts for the phase relaxation (where we use[4] κ = 1 in Eq. (9)) and the second for instantaneous diffusion, where $k \cong 1$ μs^{-1}mM^{-1} for nitroxides. G is method-specific [48] (and defined below in the next section), and for the pulse sequences defined above it is ca. 0.14 in DEER and ca. 0.52 in DQC. We also include the spin-labeling efficiency, x, which modifies the fraction of both spins that need to be flipped in PDS, showing its strong effect on the outcome of an experiment. Below we assume complete labeling for convenience in the discussion (x = 1). $K(f,T_1) = 1 - \exp(-1/fT_1)$ gives the ef-

[3] Note that the factor of $n^{1/2}$ in Eq. (9) accounts for the effective averaging of each data point. But the raw signal can be processed in several ways in order to determine distances and the distributions in distances, when possible. In [48] the number of points was not included in the expression for the SNR, because their sensitivity analysis was conducted within the context of the maximum measurable distances. In that case, based on consideration of spectral analysis (i.e. by FT), there should be at least $n_{min} = 4t_{max}/T_{dip}$ sampling points in order to satisfy the Nyquist criterion for the highest dipolar frequency of the Pake doublet, $2\omega_d$ (and just 2 for $t_{max} = T_{dip}/2$). It is this n_{min} that should be used as N in Eq. (9) to estimate the SNR for the dipolar spectrum in the frequency domain. Oversampling does not degrade the SNR, which is determined by the total number of signal samples (ft_{exp}) and n_{min}, but it helps to reduce aliasing in the spectrum and may have other positive effects. For reliable recovery of distributions in distances by Tikhonov analysis, 50–100 data points are desirable with the SNR in the data record of at least 30 [72, 91] Eq. (9) thus gives a conservative estimate.

[4] When κ > 1, e.g. for relaxation effects from nuclear spin diffusion, its partial refocusing in the DQC experiment provides an improved SNR [19].

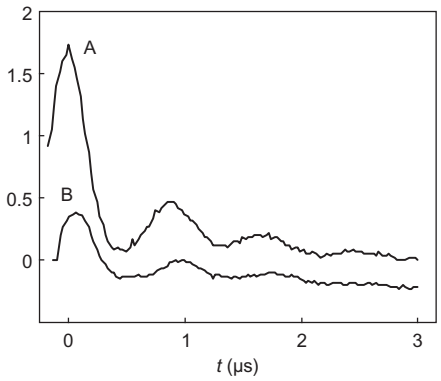

Fig. 8. Comparison of the DQC signal vs. t_ξ (A) and 4-pulse DEER vs. t (B) operating at 17.4 GHz for spin-label at position 340 in the cytoplasmic domain of band 3 protein [35]. The same resonator and sample was used in both cases, data collection time was 25 min, T was 70 K. In DQC 9 ns π-pulses, (i.e. 20 Gauss B_1) were used; 16/32/32 ns observing pulses and 28 ns pumping pulse were used in DEER. SNR of DQC is 142, in DEER it is 43. The DQC SNR may be improved by using shorter π-pulses. An additional advantage of DQC was due to its partial cancellation of nuclear spin diffusion. (Current operating performance for these conditions yields SNR's that are greater by a factor of 2.5.) (Unpublished data, the protein courtesy of Zheng Zhou.)

fect of incomplete spin-lattice relaxation for a given relaxation time, T_1 and repetition rate, f. (K is 0.72 for the optimal repetition rate, when $fT_1 = 0.79$ and is unity when $fT_1 << 1$.) As an illustration of the capability of PDS in various regimes, we consider the following examples: fully supported by experiment.

a) Short distances, low concentrations

For a short distance of 20 Å ($T_{dip} \equiv \nu_d^{-1} = 154$ ns), we set $t_{max} = 0.48\ \mu s \approx 3T_{dip}$ in order to provide very good resolution of distance; T_m is taken as 1.0 μs, i.e. the shortest within its typical range; 8 ns steps in t yielding 60 data points are taken as producing the signal record; a pulse repetition frequency f of 1 kHz should be optimal for a spin-labeled protein at 70 K. One finds from Eq. (9) that just $t_{exp} \approx 4$ min of signal averaging of the DQC signal provides a SNR of 10 for a C of 1 μM. DEER will require nearly one hour (50min) to achieve this result. Note that this concentration corresponds to just 10 picomoles of protein. A high SNR of 100 for DQC could be attained in 6.5 hours for the same amount of protein.

b) Long distances

We assume $t_{max} = 4$ μs, a typical T_m of ca. 2 μs, and the steps in t are taken to be 50 ns. Then a SNR of 10 will be reached in 8 h for a C of 2.1 μM for DQC (while for DEER it would be 104 h). By using one period of T_{dip} we find $R_{max} = 59$ Å; for half of the period, R_{max} is 75 Å. (Longer distances cannot be estimated reliably with this SNR). An accurate analysis of the distance distribution requires a higher concentration of at least 10 μM in order to provide a SNR of at least 50 [72, 91], under otherwise similar conditions.

c) Distances in the optimal PDS range

We consider 50 Å as an upper limit for the 'optimal' PDS distance range. T_{dip} is then 2.4 μs, therefore a t_{max} of 2.4 μs suffices to provide the distance sufficiently accurate for a structure constraint. We assume the rather challenging case of $T_m = 1.5$ μs; steps in t are taken to be of 32 ns; f is 1 kHz, C is taken as 25 μM; but now we require a good SNR of 50. Such a SNR will be achieved in 16 min by DQC. DEER will require nearly 3.5 hours to achieve the same result, or else the concentration must be increased (by a factor 2–4). Shorter distances of 20–45 Å are measured faster, or else yield a better SNR or resolution.

Absolute spin sensitivity is closely related to the concentration sensitivity; however it does increase rapidly with an increase of the working frequency due to the smaller volume of a resonator used at a higher frequency, e.g. at Ku-band 25–250 picomoles of protein are routinely used in the optimal distance range. The smaller amounts are better suited for DQC. These amounts can be reduced by about an order of magnitude using smaller resonators than we currently employ, but by an even greater factor at a higher working frequency.

We remind the reader that the above estimates relate to our 17.3 GHz spectrometer; lower estimates of sensitivity, in particular absolute sensitivity, would apply to the typical pulse spectrometers that operate at 9 GHz, (cf. below).

8. Further Aspects of Sensitivity of PDS: Higher Frequencies

To complete our discussion of sensitivity, we address the single-shot SNR of the dipolar signal, S_1 in Eq. (9) in the absence of relaxation and the other factors considered above, with a view to estimate its frequency dependence. It is clear that this sensitivity is determined by the SNR of the relevant echo signal, which depends on the fraction of participating A spins giving rise to the echo, and is then modified with the factor (≤1) depending on the fraction of the B spins flipped by the pump pulse; (in DQC B spins are also A spins, but the approach below works similarly). The SNR is highest when all (or nearly all) spins are excited, resonator Q matches the bandwidth of the echo (and that of excitation pulses), and the signal reception is optimized, e.g. by matched filtering. The single-shot SNR of the part of the echo modified by dipolar coupling, S_1 can be estimated as (cf. [90]):

$$S_1 = \beta_0 \omega C V_s G H (Q\omega / V_c F_N \Delta f)^{1/2} \qquad (10)$$

wherein β_0 is a constant, $\omega = 2\pi f$, where f is the working frequency; C is the spin concentration in the sample; V_c is the resonator effective volume; $V_s = V_c\eta$ is the sample volume, with η being the filling factor of the resonator; G and H are the spectral excitations of A and B spins, respectively; Q is the loaded Q-value of the resonator; F_N is the system noise figure; Δf is the receiver bandwidth.

Often, Δf is set to match the signal bandwidth e.g. the spin-echo signal is integrated (usually between the points located at ca. 2/3 of the echo height). The spectral extent of the echo is proportional to γB_1, thus for optimal signal reception $\Delta f \propto \gamma B_1$. Also, Q is set to accommodate short pulses in DQC and the frequency separation in DEER. We assume $Q \propto K_1\omega/\gamma_e B_1$ with $K_1 \approx 0.1$–0.2 for DEER and $K_1 \approx 1$ for DQC. For a modern solid-state receiver, F_N only slowly degrades with frequency increase; therefore it will be considered a constant.

Both, G and H are determined mainly by the spectral coverage of the π-pulses. Thus the better spectral coverage of DQC is included in these factors. In DQC $G \approx H$, and we assume for simplicity the same for DEER, which is usually the case. For DEER, typically, the pump pulse should be in the 20–40 ns range to avoid signal distortions, yet provide adequate excitation. In DQC π-pulses can be as short as 3–4 ns. For $B_1 << B_s$ we can let for both DQC and DEER $G \approx H \propto B_1/B_s$, where B_s is the spectral extent in Gauss; so $GH = K(B_1/B_s)^2$, with K dependent on pulse method [48, 76]. In the opposite case of large $B_1 \geq B_s$, appropriate for DQC, G and H levels off approaching unity. Given practical considerations, one chooses in DQC a value of $B_1/B_s = K_2 < 1$ (e.g. $K_2 \sim 0.7$ for DQC as compared to 0.1–0.2 for DEER).

With all these considerations, the achievable SNR for the integrated dipolar signal becomes[5]

$$S_1 \propto \omega^2 C V_c^{1/2} \eta B_s^{-1} K K_2 K_1^{1/2}. \qquad (11)$$

[5] A larger number of pulses in DQC and the factor of 2 in the denominator (cf. Eq. (8)), makes the difference between the two methods less dramatic as follows from more accurate analysis.

For the same type of resonator[6] $V_c = \alpha\omega^{-3}$, with α being determined by the resonator design. Then the SNR depends on concentration as

$$S_1(C) \propto C\alpha^{1/2}\omega^{1/2}\eta B_s^{-1} K K_2 K_1^{1/2} \qquad (12)$$

whereas the absolute sensitivity in terms of number of spins, N is:

$$S_1(N) \propto N\omega^{7/2}\alpha^{-1/2} B_s^{-1} K K_2 K_1^{1/2}\,. \qquad (13)$$

For very high frequencies $B_s^{-1} \propto \omega$, so

$$S_1(C) \propto C\alpha^{1/2}\omega^{-1/2}\eta K K_2 K_1^{1/2}\,, \qquad (14)$$

$$S_1(N) \propto N\omega^{5/2}\alpha^{-1/2} K K_2 K_1^{1/2}\,. \qquad (15)$$

We assumed above there is enough power available at the higher frequencies to maintain optimal SNR, so $S_1(C) \propto \omega^{-1/2}$ and $S_1(N) \propto \omega^{5/2}$. Thus it would appear that concentration sensitivity is not benefited by going to higher frequencies (e.g. in the mm-range) but absolute sensitivity should improve. On the other hand even at W-band the spectral width growth is not as dramatic, and there are opportunities to design resonators with a larger value of α. Given the use of open Fabry-Perot resonators that have relatively large V_c, circular polarization, and other factors, such as different spectral shapes, these matters require more detailed consideration.

10. Distance Distributions

Several approaches to determine distance distributions of paramagnetic centers in solids were utilized in the early applications of DEER and related methods [40, 42, 92]. Such methods have been improved [72, 91, 93–95] and the Tikhonov regularization method [96] became a routine for extracting distance distributions from the raw or preprocessed data from both DEER and DQC.

The time-domain dipolar signal for uniform spin distributions in the sample may generally be viewed as $V_{intra}A_{inter} + B_{inter}$ (B_{inter} originates from singly-labeled molecules and free label or pairs where one of the spins does not participate). The A and B terms are removed to the extent possible; and then, what is taken to be a reasonably accurate representation of V_{intra} is subject to inverse reconstruction by Tikhonov regularization or related methods. The ideal-case problem can be represented by a Fredholm integral equation of the first kind

$$V_{intra}(t) = V_0 \int_0^\infty P(r)K(r,t)\,\mathrm{d}r \qquad (16)$$

with the kernel $K(r,t)$ for an isotropic sample (cf. Eqs. (2) and (3)) given by

$$K(r,t) = \int_0^1 \cos[\omega_d t(1-3x^2)]\,\mathrm{d}x\,. \qquad (17)$$

The inversion of the signal V_{intra} given by Eq. (16) to obtain $P(r)$, the distance distribution, is in principle achievable by standard numerical methods, such as by singular value decomposition (SVD), but it is an ill-posed problem which requires regularization methods in order to arrive at a stable solution for $P(r)$. In the practical implementation, the data are discrete and available over a limited time interval, and the actual form of the kernel $K(r,t)$ may differ from the ideal form given by Eq. (17).

Tikhonov regularization [72, 91, 95] recovers the full distribution in distance, $P(r)$. It is based on seeking an optimum $P(r)$, which tries to minimize the residual norm of the fit to the data while also trying to maximize the stability of $P(r)$ (i.e. to reduce its oscillations). The relative importance of both is determined by the regularization parameter, λ. The L-curve method [97] for optimizing λ is computationally very efficient and the most reliable to date, [91]. In the Tikhonov method the regularization removes the contributions of the small singular values, σ_i in the SVD that are corrupted by the noise by introducing the filter function,

$$f_i \equiv \frac{\sigma_i^2}{\sigma_i^2 + \lambda^2} \qquad (18)$$

which filters out those contributions for which $\sigma_i^2 << \lambda^2$. Further refinement of the $P(r)$ can be performed by means of the maximum entropy method (MEM) [72, 98], although it is computationally more time consuming. The latest versions of MEM and Tikhonov regularization permit one to simultaneously fit and remove the effects of A_{inter} and/or B_{inter} while optimizing the $P(r)$ from raw experimental data [72]. It was shown [70, 72, 91, 95] that distance distributions are recovered faithfully, from test data simulated using the ideal kernel of Eq. (17) even in the presence of significant noise (SNR of 10). However, real data departs from this ideal picture for several reasons, thus increasing uncertainty and requiring significantly higher SNR.

11. Some Technical Aspects of DEER and DQC

A preferred setup for 3-pulse DEER is based on using two independent power amplifiers (sources) for the two frequencies [40]. (And we find it beneficial in all cases.) A bimodal cavity resonator was used with this scheme in order to optimize sensitivity and reduce overlap between excitation profiles of pulses in pumping and detection modes. The gain in concentration sensitivity due to a higher Q-value and relatively large sample volume is offset by a low filling-factor. The modern approach, which is preferred for PDS, is based on using loop-gap (LGR) or dielectric resonators, which can be easily installed in commercial cryostats, providing days of continuous stable operation. Also, the sensitivity is higher and sample size can be much smaller resulting in small amounts, when this is needed.

3-pulse DEER can be successfully conducted with a single amplifier as we demonstrate (cf. Fig. 9a), but this usually necessitates using a TWTA in its linear regime, which is some 10–12 dB below the preferred saturation mode of operation. For this reason there may not be enough power at X-band to provide short pulses, but it was not a problem at Ku-band. Simultaneous application of bichromatic irradiation may also contribute a problem. On the other hand, the pulses in 4-pulse DEER do not need to be close, thereby avoiding some small but significant dead times effects in 3-pulse DEER. 4-pulse DEER thus can be readily set up with a single amplifier, and stronger pulses can be produced, leading to greater sensitivity. Pulse interaction is not entirely removed, but becomes less of a problem if the distance between the first two pulses is not too short.

Figure 9 compares 3-, 4-pulse DEER and DQC carried out in the same setup on the same sample with a single TWTA mode of operation. A better SNR in 3-pulse DEER compared to 4-pulse DEER is mostly due to the short relaxation times, T_1 and T_2 at the temperature of 200 K used. Note, that in both forms of DEER, the apparent dead time (time resolution) is limited by the pulse widths (one can see this point in Fig. 6), and thus is considerably longer than in DQC, which uses pulses as short as a few nanoseconds. DEER can be used, in principle, without phase cycling or even with incoherent pulses, (with performance degradation). However, DEER requires high instrument ▶

[6] Note that at a lower frequency V_c may be limited by available power, thus α needs to be smaller.

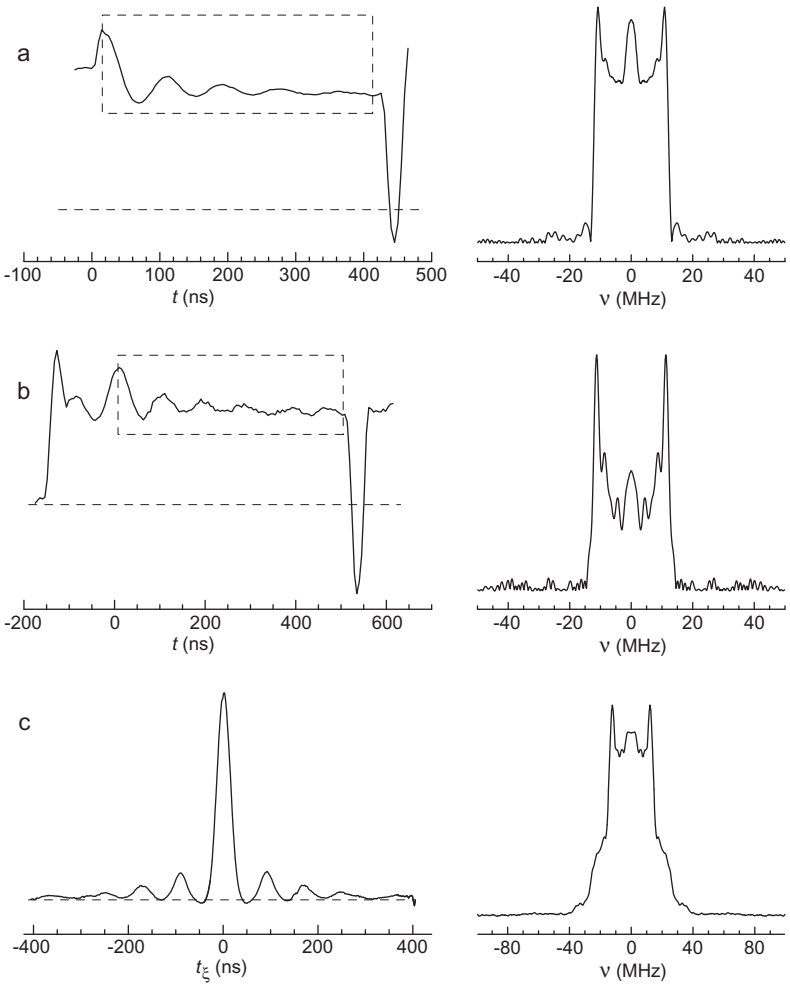

Fig. 9. 3- (**a**) and 4-pulse (**b**) DEER, and DQC (**c**) are compared for a 16.3 Å rigid biradical in LC phase V, rapidly frozen from the isotropic phase; at –80°C and 17.4 GHz. DEER was set up with a single power amplifier working in the linear regime at 10 dB below saturated output level. A low-*Q* dielectric resonator was used to accommodate the pulses at both DEER frequencies separated by ~100 MHz. π/2- and π-pulses were 10 and 20 ns in DEER and 3.2 and 6.2 ns in DQC. The pumping pulse was positioned at the low-field portion of the nitroxide spectrum. The informative parts of the signal traces in DEER are enclosed in a rectangle. In 4-pulse DEER the maximum of the signal is shifted in time as in DQC, so both 4-pulse DEER and DQC are zero dead-time pulse sequences. The outer turn-over points of the Pake doublet are missing in the dipolar spectrum from the DEER signals. The DQC signal is considerably stronger and cleaner but decays somewhat faster due to spectral broadening caused by the pseudosecular term of the dipolar coupling.[48, 62].

stability in order to maintain gain, phase, field, etc. as all small drifts directly affect the echo amplitude, leading to low-frequency noise that could limit SNR. This requires state-of-the-art pulse generation and signal detection paths with low noise and drifts, thus very high overall stability, which may be difficult to achieve in a home-built instrument, unless it is designed and built with the care given by commercial equipment vendors.

A key virtue of DQC is the suppression of the large background signal (baseline) by means of its extensive phase-cycling, in particular its use of the double-quantum filter. Unwanted modulation of the signal due to low frequency noise and drifts in phase or gain becomes less important, thereby simplifying implementation and use. This also helps to reduce nuclear ESEEM effects, which are mostly due to modulation of the large background from the single order coherence signals. The basic requirement is to provide reasonably accurate quadrature phase-cycling and sufficient B_1, which requires a more powerful and thus more expensive TWTA. Once these requirements are met, DQC is easy to set up and use. Since a higher-power TWTA could be a less attractive option for a typical user, a sound alternative is to employ minute dielectric or loop-gap resonators, which were demonstrated up to 95 GHz [99, 100], yet a cavity resonator is still a viable alternative at 35 GHz and above [100, 101].

3-pulse DEER was introduced among other things to minimize nuclear ESEEM effects, since excitation and detection regions of the ESR spectrum are well separated. For a typical 4-pulse DEER experiment with a single power amplifier at X-band, ESEEM cannot be discounted. In both DQC and DEER, standard suppression techniques are very successful, [20, 85, 102]. Also, increasing the frequency from 9 GHz to 17 GHz virtually eradicates the proton ESEEM, but deuterium ESEEM, as we find, can remain a factor in DQC.

Finally we mention orientation selection in DEER [38, 76] due to the anisotropy of the nitroxide magnetic tensors and their orientations relative to the inter-spin vector. This is an issue for DEER due to its use of selective pulses. DQC with its hard pulses is much less sensitive to orientational selectivity, but when desired orientational correlations can be revealed in considerable detail in a 2D mode [48]. The reader is referred to analyses of orientational selectivity, and its potential for distorting the dipolar spectrum in DEER by [76, 103]. However, the flexibility of side-chain spin labels, such as MTSSL, considerably decreases correlation effects. On the contrary at high fields, where orientation selection could be objectionable in standard use, it can be exploited to obtain some additional information on orientation of nitroxide side-chains, and endogenous radical centers [23, 104].

12. Summary and Perspective

In most PDS studies conducted thus far, just a few distances were typically obtained, often with the goal of detecting an important structural change or establishing the oligomerization state [15, 37, 105]. On the other hand, cw ESR routinely employs extensive protein scans [67–69] to elucidate aspects of secondary and tertiary structures. PDS is certainly capable of extensive protein mapping as we have demonstrated [18, 32]. In all, at least 70 distances (including those using WT proteins) have been obtained in our work on the CheA/CheW complex of *T. maritima*. Our approach is based on implementing triangulation to determine the ternary structure. A similar mapping effort has focused on the helix topology of α-Synuclein [18].

At present, protein structure can be reasonably accurately evaluated just using self-consistent nitroxide side-chain modeling (as noted above) and by structure refinement by CNS for a sufficiently large set of ESR dis-

tance constraints, [63]. One could anticipate that future developments will enable ESR distance restraints combined with homology modeling, nitroxide side-chain geometry simulation, and structure prediction to be applied to generate detailed 3D structures of large proteins and their complexes.

Further technical improvements are expected in PDS, in particular in DQC, which is not yet at its optimum performance. Shorter pulses with higher B_1's, better phase cycling, and resonators optimized for concentration and absolute sensitivity are expected in the near future. Both DEER and DQC will be developed at a higher frequency than Ku-band. The automation of sample processing is also planned at ACERT.

In this commentary we emphasized that PDS, as it applies to protein structure, is a rather straightforward technique in its principles and implementation, and is not overburdened with complexities. We have tried to convey our enthusiasm that PDS (both DEER and DQC) will become a standard technique for structure determination, given that it does have key virtues, which should lead to its wider acceptance.

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Notices of Meetings

We cordially invite you to participate in the 6th Asia Pacific EPR/ESR Symposium which will be held in Cairns, Australia from July 13 to July 18 in 2008 and promises to be an exciting conference.

This conference series is organised by the Asia-Pacific EPR/ESR society, with previous meetings held in 1997, 1999, 2001, 2004 and 2006. APES 2008 aims to address all aspects of EPR/ESR ranging from theoretical and experimental advances in CW EPR/ESR, pulsed EPR, high frequency and high field EPR, ENDOR, time resolved EPR, FMR, MRI, CIDEP and ODMR to applications in medicine, biology, chemistry and materials science.

More information concerning the conference will be available shortly through the APES website (www.apeprs.org)

or alternatively contact one of the organisers.

Prof. Michael Davies, CoChair daviesm@hri.org.au
Prof. Graeme Hanson, CoChair graeme.hanson@cmr.uq.edu.au
Prof. John Pilbrow, CoChair john.pilbrow@sci.monash.edu.au

The 30th EPR Symposium at the Rocky Mountain Conference

Breckenridge, Colorado, July 22–26, 2007

The conference included sessions on Spin Labeling (organized by Peter Fajer and H.-K. Shin), Metals in Neurodegenerative Diseases (organized by Graeme Hanson and Glenn Millhauser), Materials Science (organized by Pat Lenahan), and a tribute to Arthur Schweiger (organized by James Hyde and Gunnar Jeschke). The Lawrence Piette Memorial Lecture (sponsored by Medinox, Inc.) entitled "Spin Trapping of Radicals Formed in Biological Systems" was presented by Michael Davies from the Heart Research Institute, Australia. Next year's conference will be July 27–31, 2008 and will again be held in the picturesque mountain village of Breckenridge which is about a 2 hour drive west of Denver. Details will be posted at www.rockychem.com/epr/index.htm.

Sandra and Gareth Eaton

Hassane Mchaourab is having an animated scientific discussion with Eric Hustedt, Tatyana Smirnova, and Jimmy Feix. Candice Klug and Richard Mett are in the background.

TAKEN BY SANDRA EATON

The 40th Annual ESR Conference

New College, Oxford, United Kingdom, March 25–29, 2007

The 40th Annual ESR Conference took place at New College, Oxford with lectures and posters in the Inorganic Chemistry Laboratory and included joint sessions with the COST P15 action: Advanced Paramagnetic Resonance Methods in Molecular Biophysics.

The 2007 Bruker Prize Lecture by Professor Daniella Goldfarb (Weizmann Institute, Israel) described *High field ENDOR – opportunities and frustrations*. In an entertaining talk we learned that the path to high field ENDOR could be a tortuous one, but that many interesting results were obtained en route.

As this was the 40th Meeting of the ESR Group we were delighted that thirteen former Bruker Prize lecturers were able to join us. We were able to learn about the latest work of many of them.

This was a very busy meeting with nearly 200 people attending. As well as the full scientific programme we had an introductory talk on Oxford on the Sunday evening, followed by an Organ Recital in New College Chapel. On the Tuesday afternoon many participants enjoyed guided walks around Oxford.

Keynote lectures were presented by: Graham Smith (University of St Andrews) who described *The HIPER Project: sub-nanosecond pulse ESR*, Frank Neese (University of Bonn) described *Theoretical EPR spectroscopy of high-spin systems – challenges and opportunities* and Thomas Prisner (Johann-Wolfgang-Goethe University, Frankfurt) presented his lecture (generously sponsored by the British Biophysical Society) on *Structural information from decoupled spin pairs*.

40th RSC ESR Group Conference, Oxford 2007

TAKEN BY SHIRLEY FAIRHURST

From left to right: Neil Atherton (1993), Jack Freed (1990), Gareth Eaton, Sandra Eaton (2002), Jan Schmidt (1999), Dante Gatteschi (2000), Daniella Goldfarb (2007), Keith McLauchlan (1997), John Pilbrow (1998), Yuri Tsvetkov (2006), Jürgen Hütterman (2001), Klaus Möbius (1987), Klaus-Peter Dinse (2005), Wolfgang Lubitz (2003)

As well as the Keynote lectures we had a series of excellent invited and offered short talks, a poster session and the Bruker Lecture and JEOL student talk session.

This year there were 89 posters and one was selected to win the poster prize with the traditional bottle of whisky going to Esther Fischbach (Fritz-Haber Institute, Berlin), for her poster on the *Adaptation of W-band spectrometer to UHV conditions*.

The JEOL prize medal attracted a large number of excellent applications from which three were selected to present their talks. The JEOL prize medal for the best oral presentation by a young scientist was awarded to Sharon Ruthstein (Weizmann Institute) for her talk: *Characterisation of nanostructures at equilibrium and during the synthesis of mesoporous materials by DEER*.

Joint runners-up were Chris Rodgers and Olivier Rival (both from the University of Oxford). All the student talks were of a very high calibre. The three students were also presented with cash prizes by Peter Meadows (JEOL).

This year we were delighted to welcome the International EPR/ESR Society who held their Annual Meeting on the Monday evening (see also p. 3). This was the first time that this meeting has been held outside the USA.

Professor Wolfgang Lubitz (IES President) presented Professor Les Sutcliffe (Institute of Food Research, Norwich) with his certificate as a Fellow of the IES (see also EPR newsletter 17/1, p. 3).

Shirley Fairhurst

XXII Conference on Radio and Microwave Spectroscopy (RAMIS 2007)

Będlewo near Poznań, Poland, April 22–25, 2007

The traditional XXII Conference on Radio and Microwave Spectroscopy (RAMIS 2007) was held in Będlewo near Poznań, Poland, on April 22–25, 2007. The meeting was organized by the team from the Institute of Molecular Physics of the Polish Academy of Sciences in Poznań.

The conference took place in the Conference Center in Będlewo located about 30 km from Poznań, in the beautiful surroundings of the National Park of Wielkopolska. RAMIS is a multidisciplinary conference, which provides a forum for discussing the most recent advances in NMR and EPR techniques and their applications in physics, chemistry, biology, medicine, and materials science. This year we slightly changed the tradition of our meeting and beside distinguished speakers chosen among leading experts from the international scientific community we also gave younger scientists the opportunity to present their results, not only during the poster sessions but also in the oral presentations.

85 scientists participated in this meeting and were from Poland, Spain, Germany, Ukraine, Slovenia, Belgium, United Kingdom, and Romania. The scientific program consisted of an opening lecture, eleven plenary lectures, nine invited talks and 60 posters presented during two poster sessions. In the opening lecture, Piotr Pierański (Poznań University of Technology, Poznan, Poland) disclosed interesting facts about *Physics in the kitchen*. The plenary lectures presented were as follows: Stefan Jurga (Adam Mickiewicz University, Poznań, Poland) described

Conference photo, Poznań 2007

T_1 dispersion in soft matter. Miguel Moreno (University of Catanbria, Santander, Spain) talked about *Instabilities of transition metal impurities in insulators detected through resonance techniques: microscopic origin*. Franz Fujara (Technical University, Darmstadt, Germany) described the *Mechanism of proton transport in hexagonal ice*. Maya D. Glinchuk (Institute for Problems of Materials Science, National Academy of Sciences of Ukraine, Kiev, Ukraine) talked about *The peculiarities of Mn^{2+} off-center ions dynamics in $SrTiO_3$: evidence from ESR*. Janez Dolinsek (J. Stefan Institute, Ljubljana, Slovenia) told us about *Quasicrystals and giant-unit-cell intermetallics studied by NMR*. Sabine Van Doorslaer (University of Antwerp, Wilrijk, Belgium) described *Pulse EPR of transition-metal containing mesoporous silica catalysts*. Eric J. L. McInnes (The University of Manchester, Manchester, U. K.) gave a talk entitled *EPR as a tool for studying paramagnetic transition metal clusters*. Danuta Kruk (M. Smoluchowskiego Institute of Physics, Jagiellonian University, Kraków, Poland) talked about *NMR and EPR as complementary sources of structural and dynamic information*. Ioan Ardelean (Technical University, Cluj-Napoca, Romania) described *Molecular dynamics under confinement in partially filled nano- and micro-structured samples: NMR investigations*. Liviu M. Giurgiu (National Institute for Research and Development of Isotopic and Molecular Technologies, Cluj-Napoca, Romania) presented a lecture entitled *EMR Investigations of iron/oxide/polypyrrole nanocomposites*. Dieter Michel (Faculty of Physics and Geosciences, University of Leipzig, Germany) described *Molecular motion of adsorbed molecules and glass transition in confined geometry* and Czesław Rudowicz (Szczecin University of Technology, Szczecin, Poland) talked about the *Framework for modelling spectroscopic and structural properties of transition ions in crystals – a primer for the electron magnetic resonance (EMR) experimentalists*.

As well as the plenary lectures, we had a series of excellent short talks presented by the young scientists, among them three PhD students.

I am pleased to say that young scientists responded in numbers to our invitation. They were the majority of the participants and presented most of the posters. Thus the meeting seems to be attractive and interesting for young scientists, who are responsible for the future of magnetic resonance.

Although this was a very busy meeting we managed to find time for a beer tasting sponsored by Lech Browary Wielkopolski from Poznań, at the bonfire.

Selected lectures will be published in *Applied Magnetic Resonance.*

The upcoming biannual meeting RAMIS 2009 will be held in an interesting place near Poznań, Poland, in April 2009. You are already warmly invited.

Jadwiga Tritt-Goc
Chairman of Organizing Committee
RAMIS 2007

11th Chianti Workshop on Methods for Biomolecular Magnetic Resonance

Vallombrosa, Italy, June 3–8, 2007

The Chianti workshop on Methods for Biomolecular Magnetic Resonance was organized by Mario Piccioli (Florence) with the chairs Ivano Bertini (Florence) and Astrid Gräslund (Stockholm). The conference comprised 43 lectures and 84 posters with a major emphasis on biomolecular applications. Sessions were held on solid-state NMR applications on membrane proteins, aggregates and fibrils and paramagnetic solids, automated structure determination methods for high-resolution NMR on biomacromolecules, NMR relaxation measurements for structural and dynamical investigations on proteins, structure determination on paramagnetic proteins, optimized strategies for data collection in multidimensional NMR, protein-protein interactions and complexes and EPR on biological systems. The EPR talks were given by Marina Bennati (Göt-

Vallombrosa monastery drawn by Thomas Prisner

tingen), Jack Freed (Cornell), Klaus Möbius (Berlin), and Thomas Prisner (Frankfurt/Main), and covered important aspects of biomolecular EPR methods and applications. As can be easily seen by the number of EPR lectures, not only the place of the meeting was shifted from S. Miniato monastery to Vallombrosa monastery, but also the ratio of EPR to NMR talks – and therefore clearly also the number of EPR scientists attending the meeting. This was mentioned not only by the EPR scientists at the conferences but also by a number of NMR spectroscopists. We hope that in the future this ratio will be more balanced again, because the well-balanced mixture of lectures (without parallel sessions!) from solid-state NMR, liquid-state NMR and EPR made this Workshop so special and valuable in the past. Nevertheless, the workshop was excellent and very exciting with its typical Italian atmosphere, plenty of time for discussions and the – as usual – exceptional smooth and professional organization by the Florence team.

Thomas Prisner

Bruker BioSpin EPR Training courses

Rheinstetten, Germany

www.bruker-biospin.com/training_epr_eu.html

CW-EPR training course: March 5–7, 2008

FT-EPR/DEER training course: March 12–14, 2008

W-Band training course: March 12–14, 2008

CW-EPR

Introduction to the basic principles of EPR and EPR instrumentation. Participants will learn how to acquire EPR spectra and how to optimize the parameters as well as to use the acquisition and evaluation software.

Topics:

- Introduction to the basic theory of EPR
- The spectrometer and its acquisition techniques
- Optimization of EPR measurements
- Automation, simulation and data transfer
- Introduction to the EPR software packages Xepr and WIN-EPR

Special Feature:

XSophe Simulation Suite Training Course

Setup of the simulation problem / Matrix Diagonalization / Single Crystal Simulation / Power Simulation / Lineshape Models

FT-EPR & DEER

Introduction to FT-EPR Instrumentation. Participants will learn to run FT-EPR spectra and to use the FT software.

Topics:

- Spin echo techniques
- Fourier transform EPR
- Xepr software package
- Pulsed ENDOR
- ESEEM experiments
- 2D EPR ESEEM
- Variable temperature expermiments
- DEER/PELDOR distance measurements

Special Feature:

MolSophe Training Course

Setup of the EPR simulation problem / ESEEM simulation / FT-EPR simulation / HYSCORE simulation

W-Band

Introduction into high-frequency/high-field EPR at 94 GHz. The participants will acquire CW-EPR data as well as pulse EPR data including cold field sweeps.

Topics:

- Introduction to W-Band instrumentation
- Running supercon sweeps
- CW-EPR • Pulse EPR
- Xepr Software Package

Notices of Meetings

Market Place

POSITIONS

The University of New Hampshire invites

The Department of Chemistry at the University of New Hampshire welcomes inquiries from PhD scientists at any rank regarding research, and graduate and undergraduate teaching opportunities, in the area of Experimental Physical or Biophysical Chemistry. Candidates with research interests in electron resonance are particularly encouraged. Facilities include Bruker ELEXSYS E500/E560 with X-band CW-ENDOR, and Varian X- and Q-band CW-EPR/ENDOR spectrometers with dispersion and absorption mode detection and temperature capability from 2–300 K. The electron resonance lab has a variety of microwave components, bridges, cavities and electronic measuring equipment for instrument construction as well as facilities for biochemical research. Inquiries should include a cover letter explaining the type of research and teaching opportunities desired, a CV, research plans and teaching goals, and should identify 3 people as references. Send to: Christopher F. Bauer, Chair, Department of Chemistry, University of New Hampshire, Durham, NH 03824 (603) 862-1550 (fax 4278), cfb@cisunix.unh.edu. Inquiries will be reviewed as they are received. UNH supports diversity and strongly encourages women and minority candidates to send an inquiry.

Research Associate

The Dartmouth Medical School has an immediate opening in the Department of Radiology for a Research Associate. We are seeking an individual with experience in tumor biology or in gene therapy. The research involves work with animals on a daily basis, tumor cell inoculations, radiotherapy/chemotherapy, tissue pO2 measurements using EPR oximetry and tissue preparation for histology. Candidate must have experience in working with rodents (mice and rats); a background in EPR spectroscopy is preferred. The candidate should have good organizational and management skills with at least one year of relevant research experience.

This position requires an MSc, a PhD in Biology is preferred. This position is a three-year term position, with the possibility of extension.

Submit complete CV, statement of experience, and the names and contact information for 3 references to: Md. Nadeem Khan, PhD, Dartmouth Medical School, 716 Vail, Hanover, NH USA 03755, fax: 603-650-1717, e-mail: nadeem.khan@dartmouth.edu.

Dartmouth Medical School is an Equal Opportunity/Affirmative Employer and encourages applications from women and members of minority groups.

Postdoctoral or Research Associate position

A position on pulse EPR at the postdoctoral or research associate level depending on qualifications is available at the CNR-INFM MDM National Laboratory, in Agrate Brianza (Milano, Italy). The research activity is related to the pulse EPR/ENDOR investigation of impurities in semiconductors for quantum information processing. The successful candidate must have experience on the pulse EPR/ENDOR techniques possibly connected with the study of semiconductors or insulators, excellent knowledge of solid state physics and quantum mechanics, and good experimental skills. The position is initially for one year, but can be renewed up to five years. For additional information **please contact:** Prof. Marco Fanciulli, marco.fanciulli@mdm.infm.it, tel. +390396036253 (direct), +390396037489 (secretary).

Research Assistant Professor or Research Associate

Immediate openings (4) at Dartmouth Medical School in the Electron Paramagnetic Resonance (EPR) Center for the Study of Viable Systems for Research Assistant Professor (2) and Research Associate (2). For the Research Assistant Professor positions a PhD is required with expertise and experience in EPR instrumental development and/or microwave engineering. The selected individuals should be capable of independently carrying research developments that are consistent with the research directions of the EPR Center and eventually should be able to secure external funding for related research. For the Research Associate positions (requires MS or the equivalent in experience) the skills needed include expertise in at least one of the following: Tumor or Cell Biologist; EPR Instrumentalist; and microwave engineering skills. Submit complete CV, statement of pertinent experience, and request 3 references be sent to: Harold M. Swartz, Dartmouth Medical School, 702 Vail, Hanover, NH 03755, fax: 603-650-1717, e-mail: harold.swartz@dartmouth.edu. *Dartmouth Medical School is an equal opportunity/affirmative employeer and encourages applications from women and members of minority groups.*

The National Biomedical Research Center for AdvanCed ESR Technology (ACERT) at Cornell University invites applications for two Postdoctoral positions

Applications are encouraged from individuals who can contribute strongly to areas of:

(1) ESR Microscopy. This position is for the further development of ESR-Microscopy to provide true micron resolution at very high spin sensitivity, and for its application to the study of small biological samples such as single cells.

(2) Pulsed ESR and Molecular Dynamics. This position is for the study of molecular motions of membranes and proteins by multi-frequency 2D-FT-ESR techniques at 9, 17, 35, and 95 GHz. Experience in pulsed ESR techniques and/or ESR spectral simulation is highly desirable.

Interested qualified candidates should direct their inquires to acert@cornell.edu. Applicants should provide a cover letter and most recent CV. Two or three letters of recommendation are also required. Additional information about the ACERT may be found at www.acert.cornell.edu.

Director, Electron Magnetic Resonance program

The National High Magnetic Field Laboratory in Tallahassee, FL is seeking a senior researcher in electron resonance to lead the existing EMR program. The program currently comprises four faculty-level in-house scientists who develop high field instrumentation, assist the external users, and develop their own research interests. In addition to the in-house research and outside collaborations, there is strong interaction with the EPR laboratories at Florida State University (FSU) and the University of Florida (UF) in the areas of structural biology, chemistry, physics, material science and computation. The EMR program features unique high-frequency spectrometers (up to 800 GHz) and access to uniquely high magnetic fields (up to 45 T). Research focus includes nano-scale magnets, metallo-proteins, and instrument and technology development for high-frequency, time-domain EMR. Other opportunities include the use of a unique THz-Infrared light source currently under design for installation at the NHMFL in the five- to ten-year timeframe.

Minimum qualifications include a PhD in Physics, Chemistry, Biology or related. The successful candidate has a track record of outstanding scientific scholarship, and is expected to define and develop a multidisciplinary long-term vision for the program.

Senior scientists with a strong international reputation, strong publication and grantsmanship records are encouraged to apply. Particular research interests in one or more of the following areas is preferred but not required: molecular magnets and other nano-scale magnetic material and/or metalloprotein structure and function. The appointment will be either at the Scholar Scientist level (non-tenure track) in the NHMFL or a Professorial position in an appropriate academic department at FSU.

To apply, please send your CV, cover letter describing your experience, and names and contact information of 3 references to Professor Peter Fajer, Chair, EMR Director Search Committee, National High Magnetic Field Laboratory, Florida State University, 1800 E. Paul Dirac Drive, Tallahassee, FL 32310-2740, 850-645-1337, fax 850-644-1366; or e-mail fajer@magnet.fsu.edu. The selection started on April 15 and will continue until position is filled. *An Equal Opportunity/Access/Affirmative Action Employer.*

Postdoctoral positions at Davis Heart and Lung Research Institute, The Ohio State University

(1) A position for a scientist with experience in magnetic resonance instrumentation development and application. The candidate should have experience in EPR/MR hardware or software development and applications to chemical or biological systems. Salary commensurate with experience. Please reference PA06 in your application.

(2) A position for a scientist with experience in cardiac NMR spectroscopy or imaging research to perform isolated heart and in vivo studies of alterations in myocardial energetics and metabolism in the postischemic heart. Salary commensurate with experience. Please reference PA07 in your application.

Send CV to: Dr. Jay Zweier, 473 West 12th Avenue, Room 110, Columbus, Ohio 43210 or zweier-1@medctr.osu.edu. *The Ohio State University is an equal opportunity/affirmative action employer. Qualified women, minorities, Vietnam era veterans and individuals with disabilities are encouraged to apply.*

Postdoctoral position at Physics Department, National Dong Hwa University, Taiwan

A postdoctoral position is available in the laboratory of Prof. Shyue-Chu Ke at the Physics Department, National Dong Hwa University, Taiwan. The research will involve application of EPR and pulsed EPR spectroscopy to understand the fundamental questions related to adenosylcobalamin-dependent enzymatic reactions. Additional information about the laboratory is available at: www.phys.ndhu.edu.tw/teachers/ke/ke.htm. Applicants should have experience in analytical techniques and continuous or pulsed EPR methods and data analysis. Experimental physical chemist with experience in cell culture or synthesis would be beneficial, but is not essential. The position is available this summer and appointments are for up to 3 years. If interested, please send a CV and summary of previous research experience to ke@mail.ndhu.edu.tw.

Faculty position for an EPR scientist

The Division of Cardiovascular Medicine, Brigham and Women's Hospital, is seeking a research scientist with experience in electron paramagnetic resonance to fill a faculty position. The candidate should hold a PhD and/or MD degree with at least 2-3 years postdoctoral research experience. The successful candidate is expected to establish a research program in collaboration with faculty members interested in the role of oxygen and reactive oxygen species in cardiovascular physiology and disease using EPR technology. This position offers an attractive start-up package including appropriate instrument support.

Interested applicants should submit a CV, a brief statement of research interests, and the names of 3 references to: Linda Johnson, Brigham and Women's Hospital, 221 Longwood Avenue, Room 247, Boston, MA 02115 or ljohnson@rics.bwh.harvard.edu (subject: EPR faculty search). *Brigham and Women's Hospital is an equal opportunity employer. Women and minority candidates are particularly encouraged to apply.*

EQUIPMENT

Design and construction of EPR electronics

The University of Denver can supply electronic design and construction services for EPR applications. Low-noise pulse amplifiers, low-noise 100 kHz preamplifiers, boxcar integrators, and pulse timing systems are available. We also supply a conversion kit to convert Varian field-control units to voltage-controlled scan operation. A 6-digit 1-ppm frequency counter is available in X-, C-, S-, L-band, or MHz versions. Complete microwave/RF bridges from 150 MHz to L-, S-, or C-band are available from designs previously built and tested at the University of Denver.

Please contact: Richard W. Quine, e-mail: rquine@du.edu, phone: 1-303-871-2419

Available: EPR accessories and supplies

We have some excess EPR accessories and supplies that might be of use to other labs. For example, we have a lot of chart paper, pens and ink for older recorders, and some spare parts and accessories such as VT Dewars for older spectrometers. If you need something for an older-style Varian or Bruker spectrometer, ask us – we might be able to help. Most items are available for shipping costs.

Gareth R. Eaton geaton@du.edu

For sale: Varian equipment

Resonance Instruments has available: (1) Replacement klystrons for Varian EPR bridges (at reduced prices) and other klystrons. (2) Varian V4500-41A low/high power microwave bridge with new klystron – excellent condition. For more information **please contact:** Clarence Arnow, President, e-mail: rii1@earthlink.net, phone: 1-847-583-1000, fax: 1-847-583-1021.

Available: Used Varian EPR equipment

(1) Varian E-104 EPR spectrometer with vertical style bridge and e-line fieldial. (2) Varian E-9 EPR spectrometer. Both available with warranty and continued service support. (3) Varian TM cavity with flat cell holders and flat cells. (4) Varian E-257 variable temperature controller with heater sensor and insert holder. (5) Varian E-272B field/frequency lock accessory.

Please contact: James Anderson, Research Specialties, 1030 S. Main St., Cedar Grove, WI 53013, USA.

phone/fax: 1-920-668-9905

e-mail: janderson36@wi.rr.com

For Sale: ENI rf amplifieres

ENI 3200L, ENI 3100L, ENI 320L, ENI 500L. **Please address inquiries to:** eprequipment@chem.rochester.edu.

Design, upgrade and repair of EPR equipment

St.Petersburg Instruments (Russia) has available: (1) Compact high performance X-band EPR Spectrometer. (2) Microwave X-band low-noise Gunn oscillators. (3) Small low-weight magnet systems based on electromagnets or permanent magnets. (4) PC control electronic units. (5) Specialized EPR software. **Please contact:** Valeri Drapkin, St. Petersburg Instruments, P.O.Box 123, St.-Petersburg, 194156, Russia.

phone/fax: +7-812-234-25-96

site: www.spin-inc.ru

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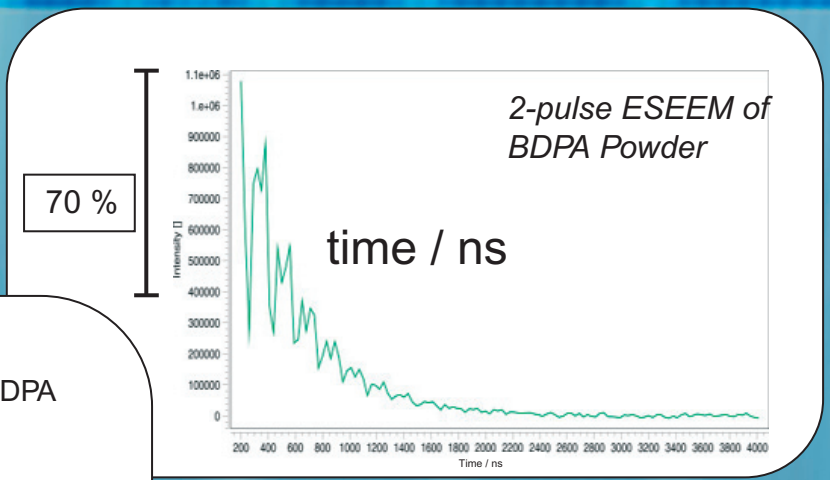

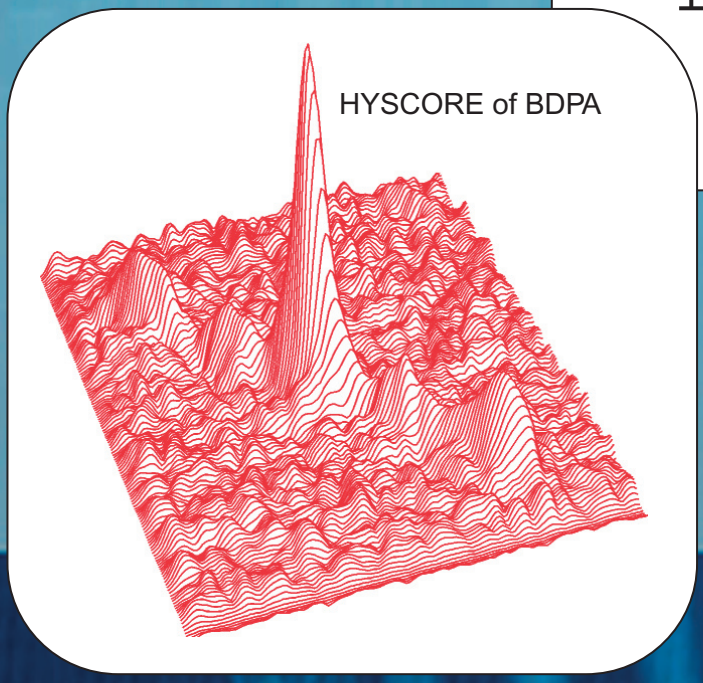

Specifications:

- ➢ 3.4 – 3.8 GHz
- ➢ 300 mW CW power
- ➢ 40 W pulse power
- ➢ Flexline probe with 30 ns π/2-pulse

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- **Highest Fidelity Signal and Field Control**
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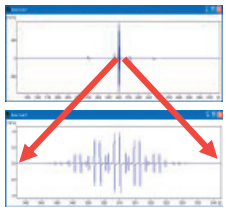
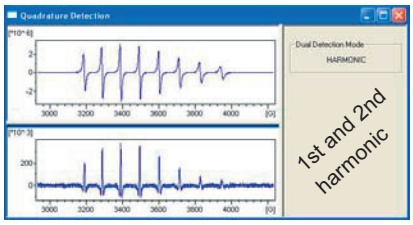


Ultra high resolution field sweep
5000 G sweep, 200000 points, 25 mG resolution



think forward

EPR Solutions