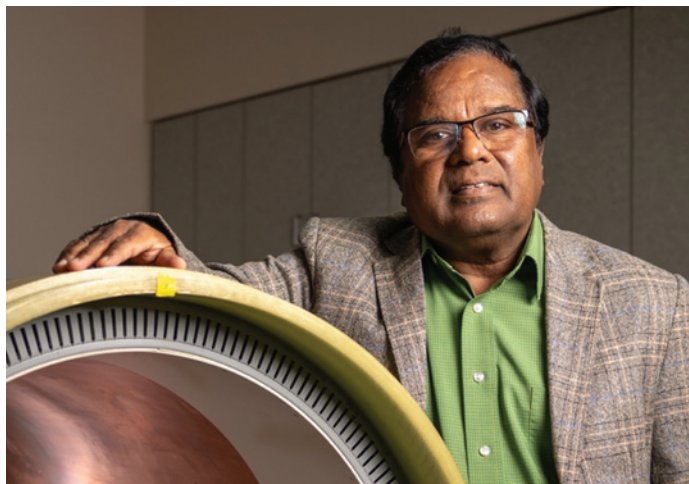


Making the Invisible Visible

A Journey from Free Radicals to Clinical EPR Oximetry



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Early Inspiration: Discovering the Invisible Through EPR

I still remember the first time I saw an EPR spectrum. It was during my PhD (1980–1985) at the Indian Institute of Technology, Madras. At the time, it appeared as little more than a set of lines – signals from an unpaired electron. I did not yet appreciate its full significance. But something about it stayed with me. There was a quiet sense that I was observing a phenomenon that revealed a deeper layer of reality – one that could not be seen directly but could be understood through careful measurement. In retrospect, that moment quietly set the course of my scientific life.

My early training in chemistry at the University of Madras immersed me in physical chemistry and spectroscopy. Those formative years shaped how I approached problems – grounding me in thermodynamics, molecular structure, and quantum principles. Yet EPR stood apart. It was not simply another spectroscopic method. It offered a unique perspective – a way to detect species that were otherwise invisible but fundamentally important. Free radicals, transition metals, and eventually oxygen itself became part of this invisible landscape. I began to appreciate that what could not be seen often mattered most!

Even then, a question began to form: could this technique, rooted in physics and chemistry, be extended into biology – and perhaps into medicine? At that stage, I did not yet know how such a transition could occur. But the question persisted, quietly shaping my thinking and guiding my decisions in the years that followed.

Formative Turning Point: Shaping a Scientific Vision

My postdoctoral training at the NIH and Johns Hopkins University (1986–1989) marked a defining turning point in my career. It immersed me in an interdisciplinary environment where the emphasis shifted from technique to meaningful biological questions. This experience

reshaped my approach to science – from focusing on methods to addressing problems of consequence.

During this time, I was influenced – both directly and indirectly – by pioneers such as James Hyde and Gareth Eaton, whose work helped define modern EPR instrumentation and methods. Their contributions demonstrated how advances in instrumentation can expand the boundaries of scientific inquiry. Equally important was the culture of curiosity, where discussions extended beyond techniques to broader biological and clinical questions. I came to understand that the value of a method lies not in its sophistication, but in its capacity to reveal new insight.

This realization transformed my view of EPR – from a spectroscopic tool into a bridge connecting the physical sciences with biology and medicine.

I have also been fortunate to be guided by exceptional mentors, peers, and collaborators. Prof. P. T. Manoharan shaped my early interest in EPR spectroscopy and scientific inquiry. Dr. Jay L. Zweier's mentorship directed my work toward biologically and clinically relevant cardiovascular problems. Dr. Murali C. Krishna has been a valued peer whose insights and collegial exchanges have consistently enriched my work in the area of cancer research. Collaborations have been equally important: Prof. Aharon Blank has contributed significantly to advances in instrumentation and translation, while Prof. Kalman Hideg's expertise in spin probe development has been central to several key aspects of my studies on molecular therapeutic. Together, these influences reflect a broader truth: scientific progress is a collective endeavor, built on shared ideas and sustained collaboration.

Defining the Central Problem: Oxygen in Biology and Disease

My early research focused on free radicals and oxidative stress – fields that revealed the dynamic chemistry underlying biological systems. These studies highlighted how reactive species influence cellular signaling, damage, and disease progression. Over time, however, my attention shifted toward oxygen.

Oxygen is deceptively simple but unique; a ground state triplet, a paramagnetic molecule! It is essential for life, yet its distribution within tissues is highly heterogeneous. In cancer, regions of low oxygen – tumor hypoxia – play a decisive role in determining treatment outcomes. Hypoxic tumors are more resistant to radiation therapy and are often associated with more aggressive behavior. Despite its importance, oxygen has remained difficult to measure directly in living systems. Most available methods are indirect or lack the resolution needed to capture dynamic changes.

EPR offered a unique solution. Because paramagnetic molecular oxygen physically interacts with paramagnetic probes through collisions to influence spectral linewidth, it can be measured directly and quantitatively. This concept – linking a physical interaction to a biological parameter – was both elegant and powerful. At this point, my research direction became clearly defined. Oxygen measurement was not merely a technical challenge; it was an opportunity to address a fundamental problem in biology and medicine.

Bridging Disciplines: From Cardiovascular physiology to Cancer

My early interest in reactive oxygen species (ROS), particularly in the context of myocardial infarction, played a pivotal role in shaping the direction of my research. Studies of oxidative stress during ischemia and reperfusion highlighted the complex interplay between oxygen availability, radical formation, and tissue injury. These investigations made it clear that understanding disease processes requires not only

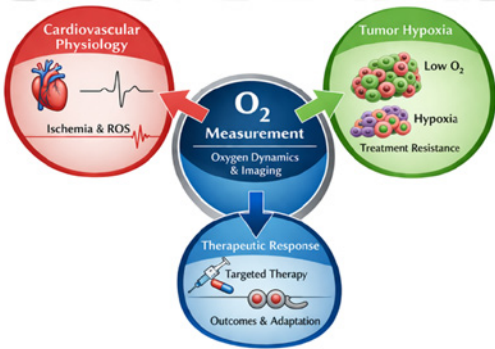


Figure 1. Oxygen as a unifying axis in my research – technology development for oxygen measurement, bridging biology and medicine.

detecting reactive species but also quantifying the underlying oxygen dynamics that drive them.

This realization led naturally to the development of EPR-based imaging and oximetry approaches, allowing us to move from indirect assessments of oxidative stress to direct, quantitative measurements of oxygen and redox status in living systems. Importantly, the conceptual framework established in cardiovascular research – linking oxygen gradients, redox imbalance, and tissue response – translated directly to cancer biology, where tumor hypoxia and oxidative stress similarly govern progression and therapeutic resistance (Figure 1).

Looking back, this progression represents one of the clearest examples of “connecting the dots” in my work – where insights gained in one field unexpectedly informed and enriched another, ultimately converging into a unified perspective on oxygen as a central determinant across disease processes.

Technical Milestone: 3D EPR Imaging of the Beating Heart

Another important direction in my research involved the development of three-dimensional EPR imaging of the beating heart, aimed at capturing oxygen and redox dynamics in a physiologically active organ. This effort required overcoming significant technical challenges, including motion artifacts, rapid signal acquisition, and synchronization with cardiac cycles.

By integrating advances in instrumentation, imaging reconstruction, and physiological control, we demonstrated the feasibility of mapping oxygen-related parameters in a moving system. This capability opened new avenues for studying cardiovascular disease models, particularly ischemia and reperfusion injury. By enabling direct assessment of myocardial oxygenation and redox changes under dynamic conditions, these approaches provided insight into tissue viability, metabolic stress, and recovery following ischemic events.

This work underscored the potential of EPR imaging to deepen understanding of cardiac pathophysiology and to inform strategies aimed at improving outcomes in ischemic heart disease.

Expanding Capability: Imaging Redox State and Tumor Oxygenation

An important component of my research has been the development of methods to image both the redox state and oxygenation of tumors in vivo. By leveraging EPR-based probes sensitive to redox changes and oxygen levels, we were able to characterize the biochemical environment of tumors beyond structural imaging.

These approaches provided insight into the dynamic interplay between oxidative stress and hypoxia – two critical factors that influence tumor progression and therapeutic response. Assessing redox status alongside oxygenation offered a more comprehensive view of the tu-

mor microenvironment, bridging molecular processes with physiological function and contributing to more targeted treatment strategies.

Building a Field: Establishing Biomedical EPR at Ohio State

My time at The Ohio State University (2002–2013) was a period of both construction and discovery. There was no established framework for biomedical EPR – only a set of possibilities. We built instrumentation specifically designed for biological applications, often developing components from the ground up.

We established experimental systems capable of supporting in vivo studies and fostered an environment that encouraged interdisciplinary collaboration. These collaborations were essential. Physicists, engineers, chemists, and clinicians each brought distinct perspectives, enabling us to address challenges that no single discipline could solve alone.

Gradually, a new field began to take shape – one that extended EPR beyond traditional spectroscopy into the realm of biology and medicine.

Demonstrating Feasibility: Direct In Vivo Oxygen Measurement

A major milestone was demonstrating that tissue oxygen could be measured directly and noninvasively using EPR. This achievement required the integration of multiple elements. Probe chemistry, instrumentation, and biological validation had to work together seamlessly. We improved probe sensitivity and stability, refined acquisition techniques, and ensured reproducibility across different experimental conditions. The result was a method capable of measuring oxygen quantitatively and repeatedly in living systems. For the first time, it became possible to monitor oxygen dynamics in vivo. This capability opened new avenues of research, particularly in understanding tumor hypoxia and its role in therapy.

Foundational Innovation: LiNc-BuO and the Path to Translation

A key advance in our work was the development of the lithium naphthalocyanine (LiNc-BuO)-based oximetry probe, which provided a robust and highly sensitive platform for measuring oxygen in biological systems. This probe exhibited exceptional oxygen-dependent line broadening with a linear response over a physiologically relevant range, while maintaining remarkable stability and biocompatibility.

Its particulate nature enabled localized implantation, allowing repeated and reliable measurements of tissue pO₂ over extended periods. The introduction of LiNc-BuO significantly improved the precision and practicality of EPR oximetry and played a central role in enabling both preclinical studies and clinical translation.

This foundational work led directly to implantable devices such as the OxyChip, establishing a clear trajectory from probe chemistry to clinical trials and patient application.

Translational Breakthrough: The OxyChip

Among the developments in my career, the OxyChip stands out as a direct link between laboratory innovation and clinical application. The concept was straightforward: an implantable sensor capable of repeated oxygen measurements. The implementation, however, required careful design and rigorous validation.

We optimized materials for long-term stability, ensured biocompatibility, and refined sensitivity. Multiple iterations were required to achieve reliable performance. The first application of the OxyChip in human studies was a defining milestone, demonstrating that EPR could move beyond the laboratory and into clinical practice.

Overcoming Complexity: The Challenge of Translation

Turning this concept into reality proved to be a formidable challenge. Biological systems are inherently complex – signals are weak, ▶

environments heterogeneous, and conditions variable. Conventional EPR instrumentation was not designed for such contexts.

We rethought nearly every aspect of the approach. Oxygen-sensitive probes had to be stable and responsive; calibration methods had to be precise; instrumentation had to function reliably *in vivo*.

Progress was incremental and often uncertain. Yet these challenges drove innovation, leading to methodologies capable of operating robustly in complex biological environments.

A Continuous Arc: Discovery to Clinical Application

Looking back, one of the most striking aspects of my work is its continuity. From fundamental chemistry to clinical studies, each stage built upon the previous one. Early investigations into free radicals and paramagnetic probes provided the conceptual and technical foundation for later advances in oxygen measurement and imaging. This progression was not always linear, but it was coherent, with each step informing and enabling the next.

Translation was not an endpoint – it was an integral part of the process. Questions that arose in clinical contexts often led back to fundamental investigations, while insights from basic science helped shape new clinical approaches. In this way, the boundary between discovery and application became less distinct, forming a continuous and evolving cycle of inquiry.

Bench to Bedside: EPR Oximetry from Mice to Men

A central theme of my scientific journey has been the deliberate progression of EPR oximetry from controlled experimental systems to real-world clinical application. What began as proof-of-principle studies in small animal models – where we established the feasibility of quantitatively measuring tissue pO_2 using oxygen-sensitive paramagnetic probes – gradually evolved into increasingly sophisticated *in vivo* investigations in tumors and cardiovascular systems (Figure 2). These early studies provided not only technical validation but also critical biological insights, particularly into tumor hypoxia and its role in therapeutic resistance. Building on this foundation,

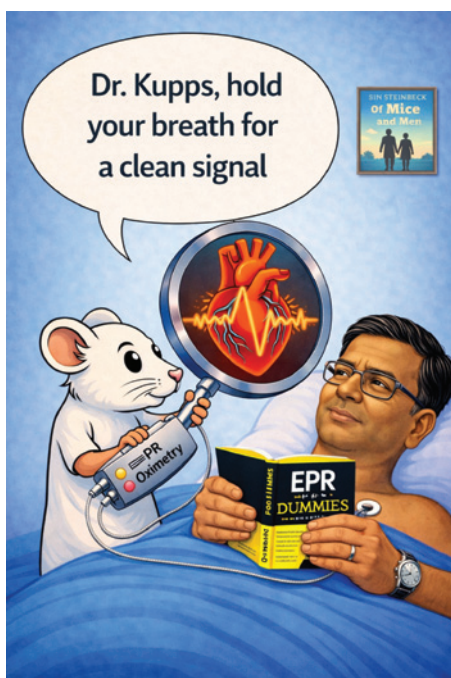


Figure 2. EPR measurements from mice to men.

we advanced probe development, instrumentation, and calibration methodologies to meet the stringent requirements of human application. This continuum culminated in first-in-human clinical studies, where implantable sensors such as the OxyChip enabled repeated, localized measurements of tumor oxygenation in patients. This “mice to men” trajectory reflects more than technological advancement – it represents a sustained commitment to translation, demonstrating that rigorous physical science methodologies can be successfully integrated into clinical research to address fundamental challenges in medicine.

Clinical Transition: Advancing EPR at Dartmouth

My transition to Dartmouth marked a shift in focus. Here, the emphasis was on applying EPR in clinical settings, moving from primarily methodological development toward direct patient-oriented research. Working closely with clinicians introduced new challenges and new opportunities – requiring not only technical adaptation, but also a deeper understanding of clinical priorities, workflow constraints, and patient needs. Could oxygen measurements be integrated into routine patient care? Could they inform treatment decisions in real time? Could they ultimately improve outcomes by guiding therapy more precisely? These questions continue to guide my work.

Clinical Impact: EPR Oximetry in Patients

At Dartmouth, we advanced EPR into clinical practice. Instrumentation was refined for patient use, with careful attention to safety, reliability, and ease of integration into clinical workflows. Implantable sensors were adapted and validated for use in human studies, enabling repeated, localized measurements of tumor oxygen over time. These efforts allowed us to move beyond single time-point assessments and begin capturing the dynamic nature of tumor oxygenation in patients. The ability to monitor oxygen longitudinally has provided valuable insights into treatment response, particularly in the context of radiation therapy and oxygen-modifying interventions. It has also opened the possibility of identifying patients who may benefit from tailored therapeutic strategies based on their tumor oxygen profile. This work represents a significant step toward establishing oxygen as a clinically actionable biomarker – one that can inform treatment planning, guide therapeutic adjustments, and ultimately contribute to more personalized approaches to cancer care.

Broad Impact: Integrating Across Disciplines

EPR oximetry has had broad impact. In cancer biology, it has improved understanding of tumor hypoxia and treatment resistance, helping to clarify why certain tumors respond poorly to therapy and how oxygen modulation might enhance treatment efficacy. In cardiovascular research, it has enabled studies of oxygen dynamics under ischemic conditions, providing insights into tissue viability and recovery following injury. In imaging science, it has contributed to the development of quantitative measurement techniques that move beyond qualitative contrast toward absolute physiological parameters.

More broadly, it has helped bridge molecular biology and functional imaging – advancing the integration of physical science methods into biomedical research and clinical practice.

Connecting the Dots: A Unifying Perspective

One idea that has resonated with me throughout my career is Steve Jobs’ observation that “you can’t connect the dots looking forward; you can only connect them looking backward”. In many ways, this perfectly reflects my scientific journey. Early work on free radicals,

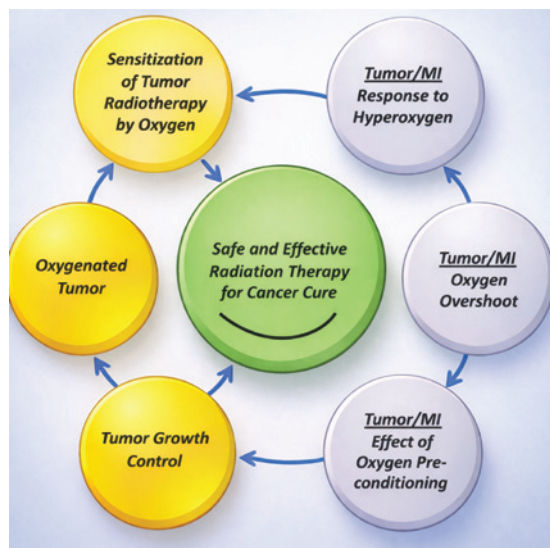


Figure 3. Connecting the dots. Convergence of cardiovascular physiology, tumor hypoxia, and treatment response into a unified framework centered on oxygen measurement.

which at the time seemed focused on fundamental chemistry, gradually led to deeper questions about oxygen – its role in biology, disease, and therapy. Years later, those seemingly separate efforts converged in unexpected ways, connecting cardiovascular physiology, tumor hypoxia, and treatment response into a unified framework centered on oxygen measurement (Figure 3). What once appeared as isolated lines of inquiry now form a coherent narrative: understanding oxygen dynamics as a common thread linking diverse diseases and therapeutic strategies. This realization has reinforced an important lesson – that pursuing important questions, even without a clear endpoint, often leads to connections that only become visible with time.

Looking Forward: From Measurement to Action

The future of EPR in medicine is promising, and in many ways, we are only beginning to realize its full potential. Efforts are underway to integrate EPR with MRI, creating complementary platforms that combine quantitative oxygen measurement with high-resolution anatomical imaging. At the same time, advances in sensor technology – particularly in implantable and minimally invasive probes – are improving sensitivity, stability, and clinical usability.

Another important direction is the development of systems capable of real-time and longitudinal monitoring during therapy. The ability to track oxygen dynamics continuously – during radiation, drug delivery, or hyperoxic interventions – opens new possibilities for adaptive

and personalized treatment strategies. Rather than relying on static measurements, we can begin to understand oxygen as a dynamic biomarker that reflects treatment response in real time.

These advances are moving us toward a broader goal: to make oxygen measurement not only feasible, but routine in clinical practice. Just as imaging modalities have become indispensable in diagnosis, quantitative oxygen assessment has the potential to become a standard component of patient management.

Ultimately, the goal is not simply to measure oxygen, but to act on it – to use this information to guide therapy, improve outcomes, and tailor treatment to individual patients. In that sense, the future of EPR lies not only in technological innovation, but in its integration into clinical decision-making.

Advice to the Next Generation: Lessons from a Translational Journey

For those entering the field, I offer a few reflections from my journey.

First, focus on meaningful problems. Technologies evolve, but impactful work is driven by questions that matter to biology, medicine, and patients. My focus on oxygen was guided by its importance, not convenience – and that decision shaped my path.

Second, be patient. Translation from discovery to application takes time. There were many periods of failed experiments and slow progress, but persistence through such phases is essential.

Third, embrace interdisciplinary thinking. Advances often arise at the intersection of fields. My work has relied on integrating chemistry, physics, engineering, and medicine, often inspired by diverse perspectives.

Equally important is resilience. Progress rarely comes from a single breakthrough; it is usually the result of sustained, incremental effort.

Finally, remain curious. Science is about asking better questions. Unexpected observations often open new directions and pursuing difficult problems – despite uncertainty – is what ultimately drives progress.

Closing Reflection: The Continuing Journey of EPR

From the first EPR spectrum to measuring oxygen in human tumors, the journey has been long and at times uncertain. Periods of doubt and slow progress ultimately shaped the work and made each advance more meaningful.

Being elected a Fellow of the International EPR Society is deeply meaningful – not as a personal milestone alone, but as recognition of the collective contributions of mentors, collaborators, and trainees. Science is never an individual endeavor.

What has remained constant is curiosity about the unseen. EPR allows us to make the invisible visible – revealing processes otherwise hidden. As technologies advance and collaborations deepen, the possibilities will continue to expand, making it both humbling and exciting to be part of this ongoing journey. ●

• NOTICES OF MEETINGS

June 28 – July 2, 2026
EUROMAR, Gothenburg, Sweden
<https://www.euromar.org>

August 2 – August 6, 2026
Rocky Mountain Conference (EPR), Snowbird, Utah, USA
<https://rockychem.com>

• NOTICES OF MEETINGS

August 29 – September 5, 2026
EF-EPR, Brno, Czech Republic
<https://www.ta-service.cz/efep2026>

September 20 – September 24, 2026
Spin Chemistry Meeting, Padua, Italy
<https://research.chimica.unipd.it/SCM2026>

• NOTICES OF MEETINGS

September 6 – September 10, 2026
HYP26, Göttingen, Germany
<https://hyp26.org>

October 18 – October 22, 2026
APES-SEST 2026, Saitama, Japan
<https://sest-nenkai.org/APES2026>