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New Champion for Chemical Analysis?

Posted By mpruess On June 27, 2008 @ 11:25 am In Feature Stories | Comments Disabled

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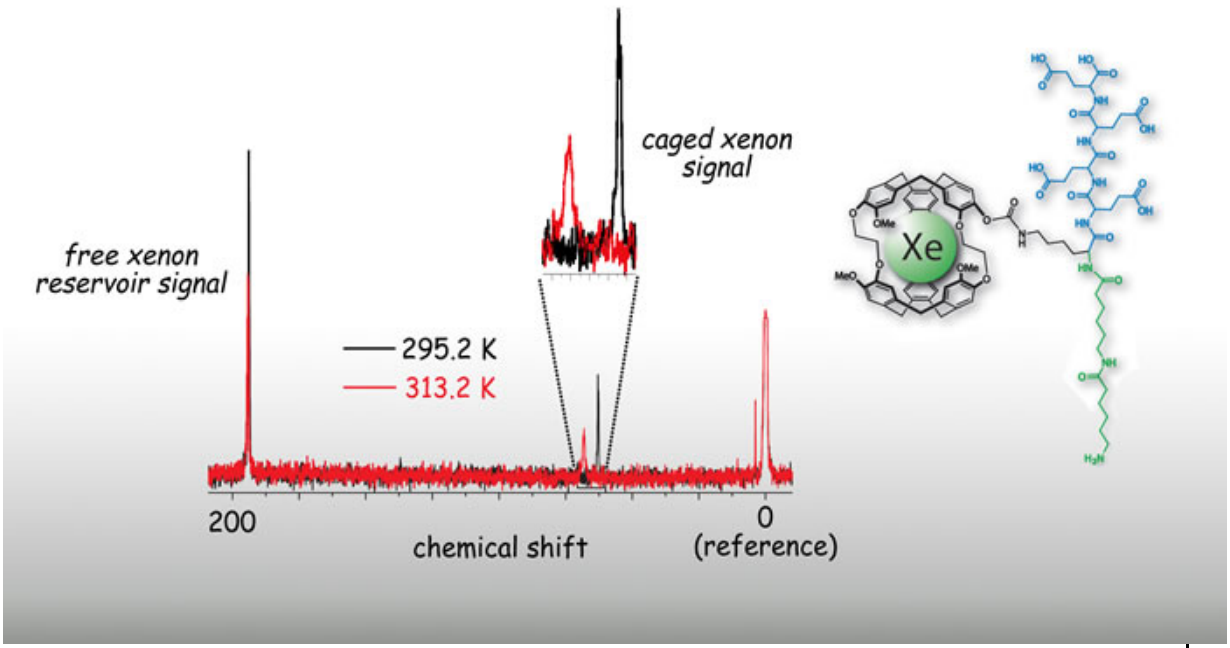

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The signal from the reservoir of abundant hyperpolarized xenon in the vicinity of the target biosensors is much stronger than that from the caged xenon. At higher temperature (red spectrum) the chemical signature of the xenon atoms inside the cages shifts, and their rate of chemical exchange through the cages increases, which also increases their rate of depolarization. The signal of depolarized xenon reentering the reservoir is easily detected as a signal decrease.

A team of researchers from the laboratories of Alexander Pines and David Wemmer at Berkeley Lab and the University of California at Berkeley has demonstrated a unique method of nuclear magnetic resonance (NMR) capable of obtaining chemical spectra, in less than two minutes, from targeted xenon biosensors in cell cultures at concentrations of mere nanomoles, billionths of a mole. The new NMR technique is several thousand times more sensitive than conventional NMR and hundreds of times more sensitive than optical analytic methods of detection.

"Using conventional NMR acquisition with nanomolar samples, achieving comparable spectra would take more than 50 years, which is about how long ago NMR was invented," says Leif Schröder of Berkeley Lab's Materials Sciences Division (MSD), who led the Pines-Wemmer team. "We have demonstrated that our technique is far better than UV-vis optical detection in this in-vitro test, and we believe we'll one day catch up with optical microscopy and radioactive labeling in biomedicine."

The researchers describe their recent results in the online edition of *Physical Review Letters*.

Extracting information from chemical samples

"UV-vis," ultraviolet-visible spectroscopy, identifies molecular groups by the wavelengths of light they absorb and the colors they reflect or transmit. A workhorse for identifying chemical compounds in biological samples and for determining their spatial distribution, UV-vis is quick, simple, and dependable, but it requires high concentrations of the target substance and can't penetrate far beneath the surface of a sample.

Nuclear magnetic resonance, by contrast, has virtually no limits to sample penetration depth, because it reports the identity and whereabouts of compounds by radio-frequency signals. Standard NMR, however, is limited by intrinsically low sensitivity.

NMR depends on the difference between the "spin up" state and the "spin down" state of target nuclei aligned in a magnetic field. When such nuclei are knocked out of alignment by a burst of radio-frequency (rf) energy, they soon realign themselves, meanwhile emitting rf signals that are highly specific to the species of the target nuclei and to their chemical surroundings.

Hydrogen is the most common target of NMR in biological systems, but even in a strong magnetic field the detectable excess in the number of spin-up versus spin-down hydrogen nuclei is only about one in 100,000. The best way to increase NMR sensitivity is through "hyperpolarization" of target nuclei, an optical technique for increasing the proportion of spin-up nuclei. Xenon, a noble gas and thus nonreactive, can be hyperpolarized to some 20-percent excess spin-up. It has the advantage of an easy-to-interpret NMR spectrum with little interference from background noise since, unlike hydrogen, it does not occur in living organisms.

Unfortunately, xenon and other hyperpolarized nuclei have, until recently, been difficult to use in biological studies for a number of reasons. To address this challenge, the Pines and Wemmer groups created xenon biosensors, chemical cages (cryptophanes) that house xenon atoms. The cryptophanes are equipped with tailored ligands that can attach to specific chemical targets such as proteins in solution on the surface of a cell.

While xenon biosensors are highly specific, their absolute numbers in biologically significant systems are extremely low compared to the number of hyperpolarized atoms in the surrounding pool of free xenon, from which the cages take up atoms one at a time; as a result, NMR signals from the cages themselves are weak.

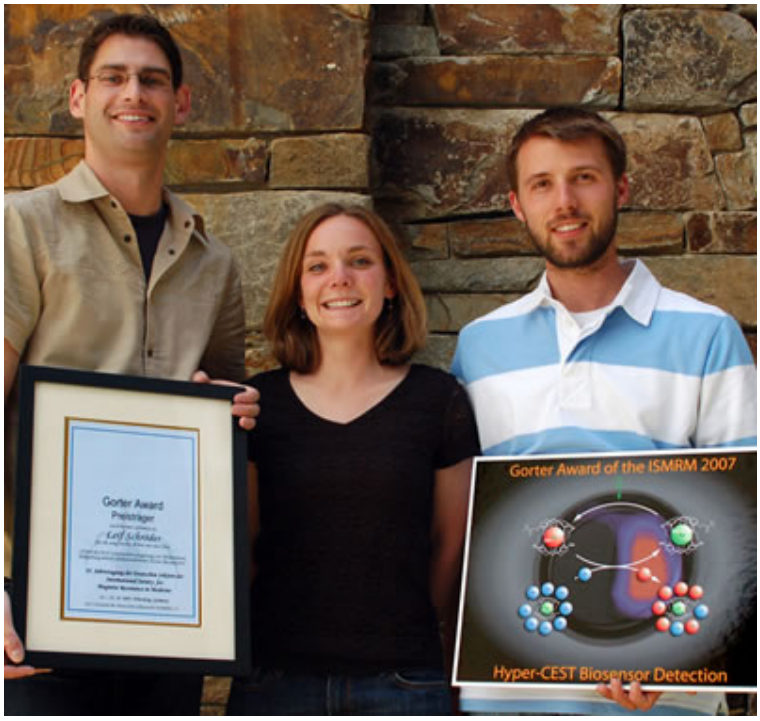
Inventing Hyper-CEST

To overcome this, the Pines and Wemmer groups invented a technique called Hyper-CEST (for "hyperpolarized xenon chemical-exchange saturation transfer"). Repeated rf bursts tuned to the caged xenon atoms, which have a unique chemical signature inside the cage, depolarize them. The depolarized xenon nuclei soon exit the cage and are quickly replaced by new polarized nuclei, which are depolarized in turn. The depolarized nuclei remain in the immediate vicinity of the target, where they are easily distinguished by contrast with the highly polarized free xenon in the surrounding pool.

The researchers have also demonstrated that the Hyper-CEST technique works well at body temperatures, a necessity for clinical applications. Moreover, through slight changes in temperature the exchange rate of hyperpolarized-to-depolarized nuclei through the cages can be regulated. These slight changes control the rates at which different kinds of cryptophane-cage hosts react with their xenon-atom guests.

Now the Pines-Wemmer team, which besides Schröder includes Tyler Meldrum of the Materials Sciences Division and Monica Smith of the Physical Biosciences Division, plus Thomas Lowery, who is now with T2 Biosystems in Cambridge, MA, has introduced further refinements that have led to the extraordinary sensitivity.

First, the team was able to calibrate the temperature sensitivity of the xenon biosensors themselves. Unlike clinical magnetic-resonance studies, most of which use the shift of hydrogen nuclei in water — only moderately sensitive to temperature changes, reducing NMR accuracy — xenon biosensors are exquisitely sensitive in their response to temperature changes.



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Leif Schröder, Monica Smith and Tyler Meldrum from the laboratories of Alexander Pines and David Wemmer were on the team that devised the ultrasensitive method of nuclear magnetic resonance that won the Gorter Award of the International Society for Magnetic Resonance in Medicine.

"We have demonstrated a temperature resolution of as little as 0.6 K," says Schröder — a little over a single degree Fahrenheit — "and we believe our method may be much more accurate."

Second, team member Meldrum devised a newly configured biosensor with a side chain, which insures that the biosensor does not readily react with the glass walls of in-vitro biological systems. This improves the resolution of NMR with, for example, cell cultures or proteins in solution.

Most promising of all is that the technique's demonstrated nanomole sensitivity makes it much more sensitive than optical techniques. A nanomole contains about 6 trillion molecules, versus the number (Avogadro's number, 6.02×10^{23}) in a mole, more than 600 sextillion. Obtaining UV-vis spectra of the same cryptophane sample used in the experiment required a minimum concentration of a millionth of a mole — a hundred to a thousand times more biosensors to obtain a comparable spectrum.

Says Schröder, "There have always been methods that show up from time to time to overcome the inherent sensitivity problem of conventional NMR detection and make the benefits of NMR available to many different applications, especially in the biosciences and medical research. We believe we have achieved a significant advance in that direction.

The research community agrees. The Hyper-CEST technique was named as a research milestone in the 40th anniversary issue of the journal *Progress in NMR Spectroscopy*, and in November, 2007, Hyper-CEST was honored with the Gorter Award of the International Society for Magnetic Resonance in Medicine.

Additional Information

- "Temperature response of ^{129}Xe depolarization transfer and its application for ultra-sensitive NMR detection," by Leif Schröder, Tyler Meldrum, Monica Smith, Thomas J. Lowery, David E. Wemmer, and Alexander Pines, appears online in *Physical Review Letters* and is available to subscribers at http://link.aps.org/abstract/PRL/v100/e257603 [4] (DOI: 10.1103/PhysRevLett.100.257603)
- More about Hyper-CEST for high-contrast MRI [5] and High-temperature Hyper-CEST [6]
- More about the design of xenon biosensors [7]
- Visit the Alexander Pines laboratory [8]
- Visit the laboratory of the Wemmer Group [9]

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[4] http://link.aps.org/abstract/PRL/v100/e257603: **http://link.aps.org/abstract/PRL/v100/e257603**
[5] Hyper-CEST for high-contrast MRI: **http://www.lbl.gov/Science-Articles/Archive/MSD-HYPER-CEST.html**
[6] High-temperature Hyper-CEST: **http://www.lbl.gov/Science-Articles/Archive/CSD-MRI.html**
[7] xenon biosensors: **http://www.lbl.gov/Science-Articles/Archive/MSD-PBD-xenon-biosensor.html**
[8] Alexander Pines laboratory: **http://waugh.cchem.berkeley.edu/**
[9] Wemmer Group: **http://www.cchem.berkeley.edu/wemmer/Home.html**