

Xenon as an NMR biosensor

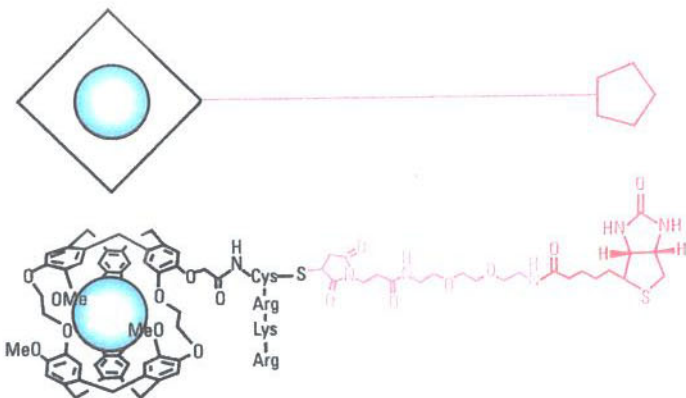
Alexander Pines and colleagues at the University of California, Berkeley, Lawrence Berkeley National Laboratory, and the Scripps Research Institute have characterized the binding of a caged-xenon sensor to its protein target by NMR. Sensors based on caged xenon could lead to the multiplexing of analyte detection in a high-density format, such as in microarrays.

Using ^{129}Xe as an NMR sensor offers several advantages. Laser polarization of ^{129}Xe increases the S/N by several orders of magnitude over nuclear-spin measurements normally made in NMR experiments. Spectra from ^{129}Xe NMR are easier to interpret because fewer lines are present and no background signal is recorded. In addition, the chemical shift and relaxation parameters of ^{129}Xe can provide information about its local environment.

Pines and colleagues formed a biosensor by caging ^{129}Xe inside a chiral cryptophane-A molecule that was modified with biotin. The caged ^{129}Xe acted as a reporter, while the modified cryptophane-A molecule interacted with biotin's binding partner, avidin.

Additional resonances appeared in the spectra when the biosensor bound to avidin. The investigators demonstrated that the signal change was due to the binding, not to the exchange rate of xenon between the free and caged forms.

Different diastereomeric forms of the cryptophane-A molecule provided



An NMR xenon biosensor consists of cryptophane-A with a caged ^{129}Xe (black) and a biotin tag (red), with a linker (purple) between the two.

distinctly different chemical shifts of ^{129}Xe when the sensor bound avidin.

Pines and colleagues suggest that sets of sensors that work in parallel could be designed on the basis of diastereomeric forms of the cryptophane-A molecule.

(*J. Am. Chem. Soc.* 2004, 126, 15,287–15,294)

Substrate for disposable microarrays

Ashish Vaidya and Michael Norton of Marshall University have prepared and characterized a surface-modified silicone elastomer for use as a substrate for DNA microarrays. The new substrate is robust and cost-effective and brings researchers one step closer to disposable microarrays.

used for microarray substrates. Even plastics have been used because they are inexpensive to produce. It is difficult, however, to immobilize DNA molecules onto plastic surfaces unless the surfaces have been modified.

Vaidya and Norton used a vapor deposition process to expose a plasma-treated silicone elastomer made of poly(dimethyl siloxane) to 3-(aminopropyl)triethoxysilane (APTS) vapors under vacuum. The APTS formed a self-assembled monolayer, which was then coupled with a heterofunctional cross-linker to produce a maleimide-functionalized elastomer. The maleimide groups on the elastomer were reacted with thiol-terminated DNA sequences. The attachment of the DNA to the maleimide groups was shown to be stable, and the surface-bound DNA was capable of undergoing hybridization with fluorescently labeled complementary DNA sequences. (*Langmuir* 2004, 20, 11,100–11,107)

Solid-state NMR of proteins

Ann McDermott and colleagues at Columbia University and Abbott Laboratories have demonstrated that protein–ligand interactions can be studied with solid-state NMR. Although solution-phase NMR has been used to characterize protein–ligand complexes, this is the first time that NMR has been used to identify protein–ligand binding sites in solid samples. The new approach significantly increases the number of proteins that can be analyzed by NMR.

Solution-phase NMR is typically limited to highly soluble proteins with low to medium molecular weights. It excludes membrane-bound proteins as well as those that are prone to aggregation. Solid-state NMR extends the application of NMR to larger proteins and those bound to membranes.

The researchers used solid-state NMR to monitor the binding of peptides and small organic molecules to a precipitate of ^{13}C -labeled protein. The protein Bcl-xL, which belongs to a family of proteins involved in regulating cell death (apoptosis), was used as a model protein. The researchers obtained high-resolution spectra that were easily reproducible, and they detected changes in the protein that occurred upon formation of protein–ligand complexes. (*J. Am. Chem. Soc.* 2004, 126, 13,948–13,953)