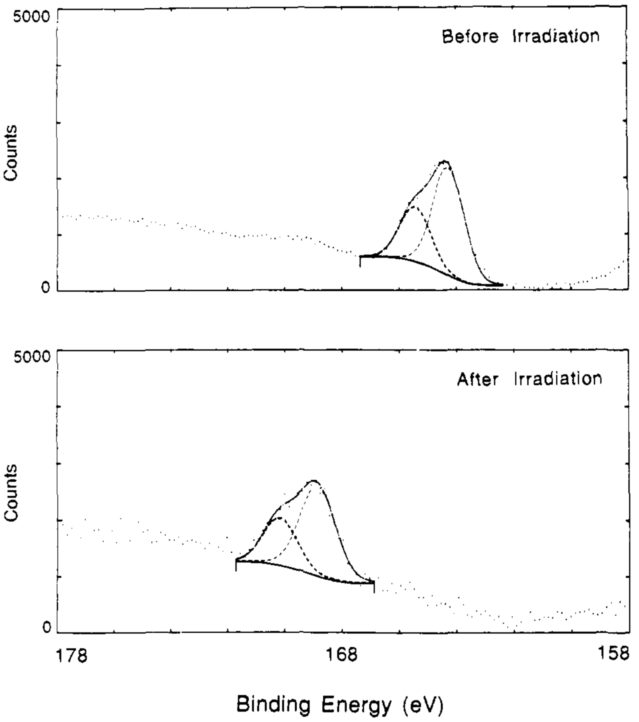

Figure 2. Oxidation of thiol to sulfonate. The XPS spectra of MTS monolayers are shown before (top) and after (bottom) irradiation with a Hg(Ar) UV light source. X-ray damage was minimized by limiting acquisition time. The take-off angle was 35°, and the operating pressure was less than 10^{-8} Torr. All spectra were referenced to the Si 2p(3/2) peak of the quartz sample, and charging problems were neutralized with a 2.7-eV electron beam.

antigen, while very little antigen was present in the areas exposed to UV light.

To characterize the film surfaces before and after irradiation, contact angle measurements and X-ray photoelectron spectroscopy (XPS) were used. Using the sessile drop method,[12] the water contact angle on an unirradiated thiol surface was 58 ± 3°. This was reduced to 30 ± 6° after irradiation. Previous work involving the irradiation of aromatic, amine, and other silane monolayers with UV light has shown that a photocleavage reaction results in contact angles of 10° or less.[13-17] XPS was used to investigate the chemical nature of the irradiated silane surface because the contact angle measurements suggested an alternate mechanism than photocleavage. Figure 2 shows the S 2p region before (top) and after (bottom) irradiation. As can be seen, the reduced thiol at 164 eV is not removed by irradiation but quantitatively converted to an oxidized form of sulfur at 169 eV. This is consistent with the conversion of the thiol to a sulfonate group.[18]

The technique outlined here for the preparation of protein patterns on a surface circumvents many of the problems encountered in earlier attempts at protein patterning,[2-4] because the irradiated thiol silane inhibits nonspecific protein adsorption. This sulfonated surface may have further applications in the protection of implants or prostheses against biofouling[19] and in the protection of critical sensor components during short-term clinical or environmental use. We have also developed a means of patterning a silane film prior to exposure of the surface to proteins which prevents any possible protein denaturation due to exposure to UV light. Such patterns may be useful for building multifunctional biosensors on a single substrate. Furthermore, because photolithography using ultrathin films has been proven to produce submicrometer patterns,[13,15] this technique should be usable to achieve patterns at much smaller dimensions.

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[12] Zisman, W. In *Contact Angles, Wettability and Adhesion*; Advances in Chemistry 49; Fowks, F. M., Ed.; American Chemical Society: Washington, DC, 1964; Chapter 1.

[13] Dulcey, C. S.; Georger, J. H.; Krauthamer, V.; Stenger, D. A.; Fare, T. L.; Calvert, J. M. *Science* **1991**, *252*, 551-554.

[14] Schnur, J. M.; Peckerar, M. C.; Marrian, C. R. K.; Schoen, P. E.; Calvert, J. M.; Georger, J. H. US Patent No. 5,077,085, 1991.

[15] Calvert, J. M.; Dulcey, C. S.; Georger, J. H.; Peckerar, M. C.; Schnur, J. M.; Schoen, P. E.; Calabrese, G. S.; Sricharoenchaikit, P. *Solid State Technol.* **1991**, *34*, 77-82.

[16] Calvert, J. M.; Georger, J. H.; Peckerar, M. C.; Pehrsson, P. E.; Schnur, J. M.; Schoen, P. E. *Thin Solid Films*, in press.

[17] Georger, J. H., Jr.; Stenger, D. A.; Rudolph, A. S.; Hickman, J. J.; Dulcey, C. S.; Fare, T. L. *Thin Solid Films*, in press.

[18] Balachander, N.; Sukenik, C. N. *Langmuir* **1990**, *6*, 1621-1627.

[19] Okkema, A. Z.; Giroux, T. A.; Grasel, T. G.; Cooper, S. L. In *Biomedical Materials and Devices*; Hanker, J. S., Giammara, B. L., Eds.; Materials Research Society: Pittsburgh, PA, 1989; Vol. 110, pp 91-96.

Far-Infrared Magnetic Resonance of Matrix-Isolated Nickelocene

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The large value of the zero-field splitting (zfs) parameter, *D*, of nickelocene (bis(cyclopentadienyl)nickel) prevents this triplet ground-state molecule from being observed by conventional electron-spin-resonance (ESR) spectroscopy. Here *D* has been directly determined in argon and krypton matrices at 4 K by far-infrared absorption in applied magnetic fields up to 4 T. *D* is customarily obtained from magnetic susceptibility (χ)[1] or inelastic neutron scattering (INS) data[2] on the solid powder or single crystal over a range of temperatures. In the case of nickelocene, a series of investigations have culminated in the thorough studies of Baltzer et al.,[3] where χ was measured on a diamagnetic host crystal (ruthenocene or ferrocene) doped with varying concentrations (up to ~6% by weight) of nickelocene. From these measurements, a value of the zfs parameter D_0 = +33.6 ± 0.3 cm^{-1} for isolated nickelocene was deduced by extrapolation to infinite dilution. Also, a value of 31.6 ± 1.0 cm^{-1} was obtained from INS measurements, but it was not considered as comparable since it was not for an isolated molecule and could also show some influence of deuterated ligands.[3]

Some time ago, Brackett, Richards, and Caughey[4] measured the far-infrared transmission spectra of polycrystalline compounds and directly obtained *D* (and *E*) for magnetic ions in molecular sites with large ligand fields. Here we have extended their procedure to study matrix-isolated molecules so as to obtain essentially unperturbed molecular parameters.

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[1] Leipfinger, H. Z. *Naturforsch. B*: *Anorg. Chem., Org. Chem., Biochem., Biophys., Biol.* **1958**, *13B*, 53-54. Nussbaum, M.; Voitlander, J. Z. *Naturforsch., A*: *Astrophys., Phys., Phys. Chem.* **1965**, *20A*, 1417-24. Prins, R.; van Voorst, J. D. W.; Schinkel, C. J. *Chem. Phys. Lett.* **1967**, *1*, 54-55. Zvarykina, A. V.; Karimov, Yu. S.; Leonova, E. V.; Lyubovskii, R. B. *Sov. Phys.-Solid State* (*Engl. Transl.*) **1970**, *12*, 385-87. Oswald, N. Ph.D. Thesis No. 5922, ETH Zürich, Switzerland, 1977.

[2] Stebler, A.; Furrer, A.; Ammeter, J. H. *Inorg. Chem.* **1984**, *23*, 3493-3500.

[3] Baltzer, P.; Furrer, A.; Hulliger, J.; Stebler, A. *Inorg. Chem.* **1988**, *27*, 1543-48.

[4] Brackett, G. C.; Richards, P. L.; Caughey, W. S. *J. Chem. Phys.* **1971**, *54*, 4383-4401.

0002-7863/92/1514-4433$03.00/0

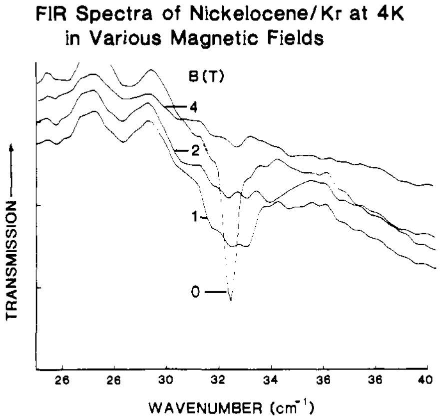

Figure 1. Far-infrared spectra of nickelocene in a krypton matrix at 4 K in various magnetic fields (*B*, in Tesla). Resolution was 0.5 cm^{-1}; each trace was for 1000 scans.

The specific details of the experimental apparatus will be reported elsewhere. It consists of an Oxford SpectroMag SM4 containing a split-coil superconducting magnet capable of attaining 5 T with a homogeneity of 1 part in 10^4 over a 2 cm diameter, but with an appendage at the lower end to allow matrices to be made in the usual way.[5] The sample was condensed on a gold-plated copper surface cooled to 4 K by a continuous flow of liquid helium. After the matrix was prepared, the gold surface was raised into the magnet, and infrared spectra were measured by reflection. IR spectra were measured with a vacuum Fourier transform spectrometer (Bruker IFS-113V) using 0.5 cm^{-1} resolution with detection in the far infrared by a liquid helium (pumped on to $\sim$1.6 K) cooled silicon bolometer (Infrared Laboratories, Tucson, AZ). $\mathrm{Ni(Cp)_2}$, purchased from Strem Chemicals and purified by vacuum sublimation, was vaporized at $\sim$50 °C and codeposited with argon or krypton gas. The rare gases were admitted at a rate of 10 mmol/h, and the matrix/nickelocene ratio was estimated to be 50–100. The deposition time was 2–3 h.

Figure 1 shows the absorption spectra of nickelocene trapped in a krypton matrix measured at zero and successively higher magnetic fields. Vibrational bands at higher frequencies agree well with those determined elsewhere[6] and are unaffected by the magnetic field. The zero-field magnetic dipole transition occurs here at 32.4 (2) cm^{-1}. A similar spectrum appears in argon at 32.5 (2) cm^{-1}, but its half-width is about 1 cm^{-1} rather than the 0.5 cm^{-1} in Figure 1, perhaps due to a more concentrated matrix. [We also observed the absorption of pure solid nickelocene condensed on the gold surface at 4 K. The zero-field transition occurred at 34.2 (2) cm^{-1} with splitting and shifted to a lower frequency at 4 T. This value is in the direction expected from the χ values of Baltzer et al.,[3] but not from the INS result.] It is significant that the values of $D = 32.5\ \mathrm{cm}^{-1}$ agree in the two matrices and presumably represent the value closest to that in the gas phase. The discrepancy of about 1 cm^{-1} from the value of Baltzer et al.,[4] which was extrapolated to eliminate nickelocene intermolecular interactions, is then probably due to a remaining matrix effect, i.e., an environment of solid ruthenocene versus rare gas, causing a slightly increased magnetic anisotropy. From our past experience with variation of the zfs of small molecules with matrix gas,[7] we would have expected a larger ΔD from the argon to krypton matrices, but apparently the sandwich molecule is more autonomous and inert to these relatively small perturbations.

In the ESR of randomly oriented molecules in a rigid medium, the spectra are dominated by the "perpendicular" lines, but near zero field these features are shifted much less than the "parallel" features by an increasing magnetic field. If only the lowest triplet Zeeman level is populated at 4 K, then for positive *D*:[8]

$$\Delta M_s = \pm 1 \qquad \Delta\nu_{\parallel} = \pm g_{\parallel}\beta B/hc \cong \pm 0.93\ \mathrm{cm^{-1}/T}$$

$$\Delta\nu_{\perp 1} = (-D/2h) + [(D/h)^2 + 4(g_{\perp}\beta B/hc)^2]^{1/2}/2$$

$$\cong +0.03 \text{ to } +0.1\ \mathrm{cm^{-1}/T}$$

$$\Delta M_s = \pm 2 \qquad \Delta\nu_{\perp 2} = 2\Delta\nu_{\perp 1}$$

Hence, the perpendicular features are shifted slightly to higher frequencies with increasing magnetic field, while the parallel features spread out symmetrically and lead to the broadening and weakening of the zero-field line, as seen in Figure 1.

Nickelocene has served as an interesting test case for far-infrared magnetic resonance spectroscopy, which we plan to apply generally to molecules with $|D| > 10\ \mathrm{cm}^{-1}$ and $S \geq 1$.

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(5) Van Zee, R. J.; Ferrante, R. F.; Zeringue, K. J.; Weltner, W., Jr. *J. Chem. Phys.* **1988**, *88*, 3465–74.

(6) See, for example: Chlor, K.; Lucazeau, G.; Sourisseau, C. *J. Raman Spectrosc.* **1981**, *11*, 183–198 and references given therein.

(7) Smith, G. R.; Weltner, W., Jr. *J. Chem. Phys.* **1975**, *62*, 4592–4604.

(8) Wasserman, E.; Snyder, L. C.; Yager, W. A. *J. Chem. Phys.* **1964**, *41*, 1763–72. Weltner, W., Jr. *Magnetic Atoms and Molecules*; Dover: Mineola, NY, 1989; Chapter III.

NOESY Studies on the $\mathrm{Fe^{III}Co^{II}}$ Active Site of the Purple Acid Phosphatase Uteroferrin

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Nuclear magnetic resonance spectroscopy is a very valuable tool for probing enzyme active sites.[1] Its application to paramagnetic metalloproteins has been hampered by the unfavorable electronic relaxation times of paramagnetic centers, which often result in relatively broad isotropically shifted ^{1}H NMR signals (typically >100 Hz).[2] Consequently, the large line widths and short T_1 values of isotropically shifted signals in paramagnetic metalloproteins were thought to render 2D experiments impractical. Recently, NOESY spectra have been successfully recorded on paramagnetic metalloproteins which exhibit relatively sharp isotropically shifted signals (<150 Hz) and thus facilitated signal assignments.[3] In our effort to gain further insight into the active

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(1) (a) Wüthrich, K. *NMR of Proteins and Nucleic Acids*; John Wiley & Sons: New York, 1986. (b) Bertini, I.; Molinari, N.; Niccolai, N. *NMR and Biomolecular Structure*; VCH: New York, 1991.

(2) Bertini, I.; Luchinat, C. *NMR of Paramagnetic Molecules in Biological Systems*; Benjamin & Cummings: Menlo Park, CA, 1986.

(3) (a) Emerson, S. D.; La Mar, G. N. *Biochemistry* **1990**, *29*, 1545–1556. (b) Busse, S. C.; Moench, S. J.; Satterlee, J. D. *Biophys. J.* **1990**, *58*, 45–51. (c) Banci, L.; Bertini, I.; Paola, T.; Tein, M.; Kirk, T. K. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 6956–6960. (d) Banci, L.; Bertini, I.; Paola, T.; Ferrer, J. C.; Mauk, A. G. *Inorg. Chem.* **1991**, *30*, 4510–4516. (e) Banci, L.; Bertini, I.; Briganti, F.; Luchinat, C.; Scozzafava, A.; Oliver, M. V. *Inorg. Chem.* **1991**, *30*, 4517–4524. (f) Bertini, I.; Briganti, F.; Luchinat, C.; Messori, L.; Monnanni, R.; Scozzafava, A.; Vallini, G. *FEBS Lett.* **1991**, *289*, 253–256. (g) Capozzi, F.; Luchinat, C.; Piccioli, M.; Viezzoli, M. S. *Eur. J. Biochem.* **1991**, *197*, 691–697. (h) Satterlee, J. D.; Erman, J. E. *Biochemistry* **1991**, *30*, 4398–4405. (i) Skjeldal, L.; Westler, W. M.; Oh, B.-H.; Krezel, A. M.; Holden, H. M.; Jacobson, B. L.; Rayment, I.; Markley, J. L. *Biochemistry* **1991**, *30*, 7363–7368. (j) Satterlee, J. D.; Russell, D. J.; Erman, J. E. *Biochemistry* **1991**, *30*, 9072–9077. (k) Banci, L.; Bertini, I.; Paola, T.; Oliver, M. V. *Eur. J. Biochem.* **1992**, *204*, 107–112.

0002-7863/92/1514-4434$03.00/0