Figure 3 shows the $^{13}C\{^{15}N\}$HSBC spectrum of [26% ul-^{13}C, 95% ul-^{15}N]flavodoxin from *Anabaena* 7120. A number of peaks have been assigned to specific types of ^{13}C–^{15}N connectivities, and one $^{13}C^{0}$–$^{15}N^{\alpha}$ cross-peak has been assigned to a specific dipeptide. The cross-peaks labeled Q-$\delta\epsilon$ (178.4 ppm ^{13}C, 113.1 ppm ^{15}N and 178.7 ppm ^{13}C, 114.2 ppm ^{15}N) have been assigned to glutamine side-chain amide groups. These are distinguished from backbone amides by two kinds of information: (a) peaks at the corresponding ^{13}C chemical shifts in the $^{13}C\{^{13}C\}$ double quantum correlated [$^{13}C\{^{13}C\}$DQC] spectrum[11] are identified as belonging to side-chain carbonyls in either aspartic acid or glutamine residues; (b) the corresponding ^{15}N peaks in the ^{15}N INEPT spectrum show modulation behavior consistent with ^{15}N nuclei bound to two ^{1}H nuclei.[6] The ^{13}C chemical shift of the peak labeled G? (170.4 ppm ^{13}C, 113.3 ppm ^{15}N) is assigned to a glycine spin system on the basis of $^{13}C\{^{13}C\}$DQC data; hence the $^{13}C\{^{15}N\}$HSBC cross-peak is assigned to coupling between a glycine $^{13}C^{0}$ and the ^{15}N amide of the next amino acid residue in the protein. A similar argument was used to assign the peak labeled GL (170.5 ppm ^{13}C, 131.2 ppm ^{15}N) to coupling between the $^{13}C^{0}$ of a glycine and the amide ^{15}N of the next amino acid residue. The amide ^{15}N was assigned to a leucine residue by identifying the single $^{1}H\{^{15}N\}$-HSBC cross-peak at this ^{15}N frequency[6] as belonging to a leucine spin system on the basis of $^{1}H\{^{1}H\}$ correlation spectroscopy (not shown). Thus the $^{13}C\{^{15}N\}$HSBC cross-peak corresponds to a glycyl-leucyl dipeptide in the flavodoxin. Since the amino acid sequence for this protein has not been determined, the dipeptide cannot be assigned to a specific position in the sequence.

The present results show the utility of the $^{13}C\{^{15}N\}$HSBC 2D NMR experiment for making sequence-specific assignments and identifying signals from side-chain amides in isotopically labeled proteins of moderate size (M_r 21 000 to 23 000). The detection of $^{13}C^{0}$–$^{15}N^{\alpha}$ bonds by 2D NMR spectroscopy will support new strategies for sequence-specific assignments that require fewer isotopically labeled protein analogues than the original dual-labeling strategy.[3,4,8] The multiple scalar coupling pathways afforded by a combination of $^{13}C\{^{15}N\}$HSBC spectroscopy with $^{13}C\{^{13}C\}$-DQC,[11-13] heteronuclear single-bond and multiple-bond $^{1}H\{^{13}C\}$ 2D spectroscopy,[13] and $^{1}H\{^{1}H\}$ 2D spectroscopy[14] form a powerful set of tools for the assignment of nearly all spin 1/2 nuclei in macromolecules of moderate size. These strategies for sequence-specific assignments are based on scalar coupling, unlike those for $^{1}H\{^{1}H\}$ 2D NMR which require detection of interresidue nuclear Overhauser effects. These new methods should prove useful in studies of proteins whose analysis by $^{1}H\{^{1}H\}$ 2D NMR methods is impractical because of their large size or spectral complexity.

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Mass Measurements Using Isotopically Labeled Solvents Reveal the Extent of Solvent Transport during Redox in Thin Films on Electrodes

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Transport of solvent during the redox reactions of thin films on electrodes has been identified as a possible influence on both the thermodynamic and kinetic aspects of their electrochemical responses.[1] A variety of methods has been used in attempts to measure solvent content of these films, including ellipsometry[2,3] and profilimetry.[4] However, those techniques which rely on measurement of thickness suffer from inability to deconvolute the contributions to swelling (or deswelling) from ion and solvent transport. Thus, the situation remains one in which speculation abounds, but accurate measurements are unavailable. In this communication, we report on the application of the quartz crystal microbalance (QCM) technique to the determination of solvent transport during redox in thin films of nickel ferrocyanide (the nickel analogue of Prussian Blue[5-12]) by comparing the difference in the total mass change (comprised of contributions from both ion and solvent transport) which results from use of isotopically substituted solvent. To our knowledge, these experiments represent the first accurate, unambiguous measurements of solvent transport in thin films on electrodes. It is especially significant that these measurements are made in the presence of simultaneous ion transport.

The QCM apparatus has been previously described.[13-15] When used in conjunction with electrochemical measurements, it allows for the simultaneous determination of minute (multilayer to submonolayer) mass changes which accompany the electrochemical reaction with a mass sensitivity of 56.6 Hz/microgram/cm^2 in the present experimental configuration. Nickel films were deposited onto the QCM gold electrode either by using a modified Watts bath[16] or by vapor deposition with identical results. The nickel ferrocyanide films were generated by using the method of Bocarsly and co-workers,[7] by maintaining the electrode potential at 1.2 V in a solution of 0.1 M KCl and 0.01 M $K_4Fe(CN)_6$ for the time required to obtain the desired film thickness. Conditions were precisely controlled to ensure uniformity of the deposited films, since this is crucial for the quantitative comparison of the QCM frequency change (which gives the mass change) to the electrochemical charge.[17] FTIR microscopy (Mattson Cygnus

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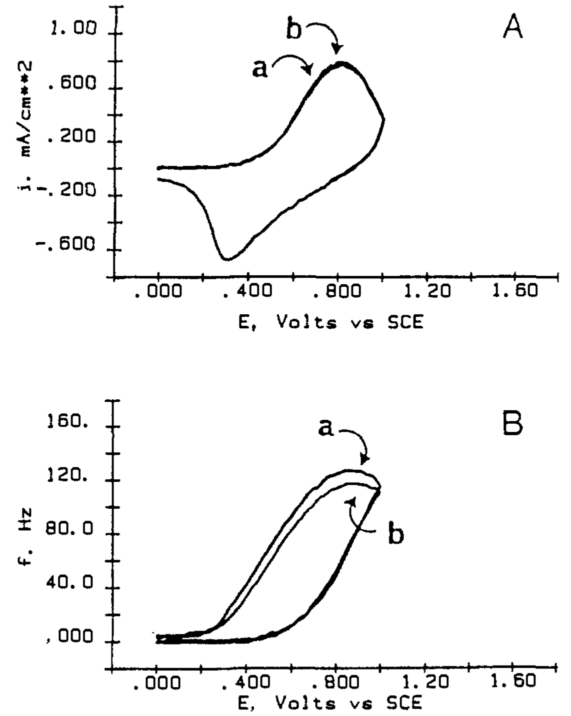

Figure 1. (A) a. Cyclic voltammogram of a nickel ferrocyanide film in 0.1 M $CsCl/H_2O$ solution. Scan rate—100 mV/s. b. Same as in (a) except that the solution contains D_2O instead of H_2O. (B) a. QCM frequency response measured simultaneously with the cyclic voltammogram in (a). b. QCM frequency response measured with cyclic voltammogram in (b).

100 with Bach-Shearer microscope) was used to verify the film uniformity. By using a 10 μm spot size, the integrated intensity of the 2090 cm^{-1} CN stretching band was measured at several points across the face of the QCM electrode, with special attention paid to the edge region. This procedure showed that the films were uniform to within experimental error. Following film growth, the electrochemical and QCM responses of the nickel ferrocyanide film were examined in 0.1 M CsCl solutions prepared by using either H_2O or D_2O. The films were electrochemically cycled for a sufficient period (usually ca. 10 min) to ensure that all of the K^+ ions from the growth solution had been exchanged for Cs^+, as judged by the attainment of a constant QCM frequency for the reduced film. No interference from K^+ was observed after this procedure.

Figure 1 shows the results of such an experiment. Curves a and b in Figure 1A show that the cyclic voltammetric response is identical in these two solutions. The measured electrochemical charges are also identical, indicating that the same number of Fe redox sites are electroactive in the two solutions. The cyclic voltammetric response shown is typical of those reported for these films in Cs^+ containing electrolytes[6,7] with the exception of a somewhat larger peak-to-peak separation, a reminder of the sensitivity of these highly structured interfaces to the method of preparation.[7] Curves a and b in Figure 1B show the QCM frequency response for these two solutions, collected simultaneously with the electrochemical data. The frequency increases during oxidation, and this increase is larger in the H_2O solution than in the D_2O solution. When the experiment was repeated in both D_2O and H_2O solutions of $CsNO_3$, identical results were obtained, providing unambiguous evidence that the observed frequency changes do not depend on the anion of the supporting electrolyte in any way.

That the frequency increases during oxidation is not unexpected, based on the earlier work of Bocarsly and co-workers.[7,8] Their results indicated that the Fe(II) state of the films contained two cations per formula unit (i.e., $Cs_2Ni[Fe(CN)_6]$), while the Fe(III) state contained one (i.e., $CsNi[Fe(CN)_6]$), so that oxidation should cause the expulsion of one cation per formula unit to maintain electroneutrality. Thus, an increase in frequency resulting from the expulsion of the cations during oxidation occurs. In addition, the dependence of the frequency change on the mass of the solvent implied some degree of solvent transport during the redox event for these films. Quantitative comparison of the QCM frequency change with the electrochemical charge reveals the extent of each.

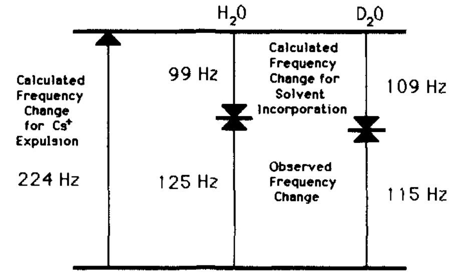

Figure 2. Schematic representation of the frequency changes measured during the cyclic voltammogram in Figure 1. See text for details.

Exactly one cation should be expelled per electron removed from the film during oxidation. Direct comparison of the electrochemical charge and the QCM frequency change revealed that the mass loss (frequency increase) which occurred during the oxidation was not sufficient to account for all of the expelled cations, i.e., a larger frequency change would have been observed if the only transport during the redox process were cation expulsion in the amount required to maintain electroneutrality. Thus, some mass must be regained by the film during or following cation expulsion to give the observed frequency change. If this mass were from solvent incorporation, then use of D_2O in place of H_2O would cause a detectable change in the observed frequency increase. Figure 2 shows a sample calculation for a typical film based on this hypothesis. Similar results were obtained for a variety of films with varying thicknesses. For this film, the calculation using the total anodic charge (2.9×10^{-3} C/cm^2) indicated that a frequency increase of 224 Hz should have been observed if the sole mass change were the expulsion of 3×10^{-8} mol/cm^2 of Cs^+. The observed frequency increases in D_2O and H_2O were 115 and 125 Hz, respectively. The discrepancies between the calculated and observed values in D_2O and H_2O were thus 109 and 99 Hz, respectively. (Given the signal-to-noise ratio of the measurement, the minimum detectable frequency difference is ca. 0.5 Hz.) If the entire discrepancy between the calculated frequency increase for Cs^+ and that observed experimentally were due to a net increase in the solvent content of the film, then the discrepancy for the D_2O case should have been 10% larger than that for the H_2O case because the molar mass of D_2O is larger than that of H_2O by 10%. This is exactly what was observed. The agreement is quantitative, and the requirement that the difference between the calculated mass gain due to D_2O and H_2O be 10% serves as an internal check of the veracity of the calculations and underlying assumptions. One such assumption is that D_2O and H_2O behave in chemically identical manners so that identical numbers of moles of each are transported during the two experiments shown in Figure 1. A most important assumption, crucial to the use of the frequency change in quantitative calculations,[14,15] is that the film behave as a rigid layer so that the frequency change may be linearly related to the mass change. The excellent agreement presented above is unambiguous evidence of this. Feldman and Melroy[18] have previously reported rigid layer behavior for Prussian Blue films. In their study qualitative evidence for solvent transport during redox was observed.

The picture which emerges for the cesium case is that during oxidation some Cs^+ ions are expelled with a consequent influx of solvent to fill the void volume left within the lattice. During reduction, the species undergo transport in the opposite direction, with complete reversibility of the mass changes. These data show that 3.2 mol of H_2O are incorporated when 1 mol of Cs^+ ions is expelled. Approximate molar volumes for the two species are 18

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cm^3/mol (from the bulk density of water) and 12 cm^3/mol (from the ionic radius of Cs^+ of 0.169 nm given by Pauling[19]), so at first glance it seems that expulsion of 1 mol of Cs^+ should allow for accommodation of only 0.67 mol of H_2O. Thus, either the effective volume of Cs^+ within the lattice is considerably larger than its ionic radius would indicate, or the effective volume of a water molecule within the lattice is considerably smaller than it is in bulk water or some combination of the two. Since it is unreasonable to postulate a water density within the lattice which is greater (by a factor of nearly 5!) than the bulk value to account for the discrepancy, we attribute the majority of the effect to a much larger effective volume for Cs^+. Geometric arguments based on the sizes of Cs^+ and H_2O in relation to the size of the octahedral cavity[10,20-22] can be made which are in agreement with this conclusion. Briefly, even though the Cs^+ cation does not completely fill the cubic cavity (which is 0.5 nm on a side[10,20-22]), it is just large enough to preclude the presence of water molecules. Thus, void space is left within the cavity which does not have the proper geometry to accommodate the incorporation of interstitial water. A more detailed description of this "discreteness of size" effect will be reported in a future contribution, along with a discussion of the insight provided by such measurements of solvent transport ito the remarkable sensitivity of the formal potential of these nickel ferrocyanide films to the identity of the presence of various alkali metal cations.

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Facile Cleavage of C–H Bonds. Reaction of Trimethylaluminum with Bis(diphenylphosphinoyl)methane: Synthesis and Molecular Structure of $[Al(CH_3)][(C_6H_5)_2P(O)CP(O)(C_6H_5)_2]_2[Al(CH_3)_2]_2$

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The ability of multidentate ligands to promote metal–metal interactions in bimetallic complexes is often considered an attractive feature in many organometallic systems. To this end, many workers have employed the bidentate phosphine ligand bis(diphenylphosphino)methane. The recent popularity of this ligand may be traced to the fact that the close proximity of the two phosphorus atoms can often facilitate interesting behavior in bimetallic complexes.[1-5] Relative to organo-main group chemistry, this ligand has also been shown to play a role in various organoaluminum catalytic systems.[6-8] Surprisingly, the corresponding transition metal and main group metal chemistry of the dioxygen analogue of bis(diphenylphosphino)methane, bis(diphenylphosphinoyl)methane ($[(C_6H_5)_2P(O)CH_2P(O)(C_6H_5)_2]$) remains largely unexplored. Herein, we report the synthesis[9] and molecular structure of $[Al(CH_3)][(C_6H_5)_2P(O)CP(O)(C_6H_5)_2]_2[Al(CH_3)_2]_2$ isolated from reaction of the bidentate ligand with trimethylaluminum in chlorobenzene. A particularly significant point about bis(diphenylphosphinoyl)methane is the fact that the hydrogen atoms of the central methylene carbon are sufficiently acidic so as to undergo Al–R/C–H bond cleavage thus resulting in the unusual organoaluminum title compound. The X-ray crystal structure of the compound is shown in Figure 1.

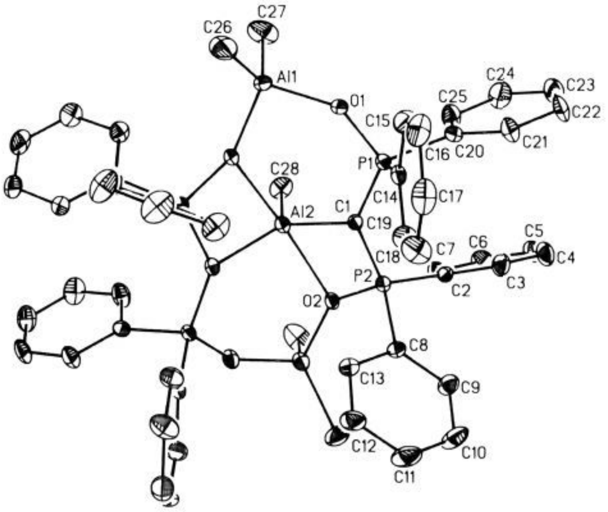

Figure 1. A view of the $[Al(CH_3)][(C_6H_5)_2P(O)CP(O)(C_6H_5)_2]_2[Al(CH_3)_2]_2$ molecule showing the atom labeling scheme. Thermal ellipsoids show 20% probability levels; hydrogen atoms have been omitted. Selected bond distances (Å) and angles (deg): Al1–O1, 1.772 (4); Al1–C26, 1.986 (9); Al1–C27, 1.977 (9); Al2–C1, 1.982 (6); Al2–C28, 1.994 (10); Al2–O2, 2.174 (4); P1–C1, 1.680 (6); P2–C1, 1.672 (6); P1–C20, 1.811 (6); P1–C14, 1.808 (7); P2–C2, 1.800 (6); P2–C8, 1.799 (7); C1–Al2–C28, 121.0 (2); C1–Al2–C1′, 118.0 (2); C28–Al2–C1′, 121.0 (2); O2–Al2–O2′, 163.2 (2).

X-ray intensity data were collected on a Nicolet R3m/V diffractometer by using an $\omega/2\Theta$ scan technique with Mo Kα radiation (λ = 0.71073 Å) at 26 °C. The title compound crystallizes in the monoclinic space group $C2/c$ with unit cell parameters a = 25.756 (4) Å, b = 10.126 (2) Å, c = 23.038 (4) Å, β = 119.54 (1)°, V = 5227.17 Å^3, and D_{calcd} = 1.25 g cm^{-3} for Z = 4. The structure was solved by direct methods and refined, based on 2104 observed reflections with intensities $I \geq 3\sigma(I)$, by using SHELXTL.[10] Least-squares refinement converged at R = 0.056 (R_w = 0.070). Anisotropic thermal parameters were used for all non-hydrogen atoms. Hydrogen atoms, located from difference Fourier maps, were placed in idealized positions with fixed isotropic temperature factors.

This laboratory has long had an interest in the interactions of organoaluminum species with macrocyclic ligands. While initially we were concerned with reaction of organoaluminum species with

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(9) Inside the drybox a reaction vessel was charged with bis(diphenylphosphinoyl)methane (5.0 mmol) and chlorobenzene (25 mL). An excess of trimethylaluminum (7.5 mmol) was slowly added via syringe. The reaction vessel was removed from the drybox and heated (100 °C) in an oil bath for several hours. The system was vented periodically to release gas which was eliminated during the course of the reaction. Upon cooling to room temperature a multitude of colorless extremely air-sensitive crystals were obtained in quantitative yield: ^1H NMR (C_6D_6) δ −1.22 (s, 3 H, Al(CH_3)), −0.93 (s, 6 H, 2 Al(CH_3)), −0.80 (s, 3 H, Al(CH_3)), −0.27 (s, 3 H, Al(CH_3)), 7.31 (m, 40 H, C_6H_5).

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