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Application of *Para*hydrogen Induced Polarization Techniques in NMR Spectroscopy and Imaging

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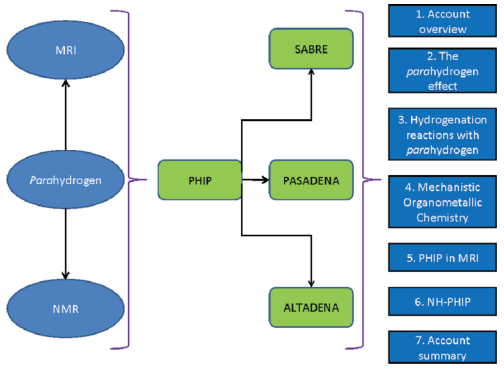
CONSPECTUS

Magnetic resonance provides a versatile platform that allows scientists to examine many different types of phenomena. However, the sensitivity of both NMR spectroscopy and MRI is low because the detected signal strength depends on the population difference that exists between the probed nuclear spin states in a magnetic field. This population difference increases with the strength of the interacting magnetic field and decreases with measurement temperature. In contrast, hyperpolarization methods that chemically introduce *para*hydrogen (a spin isomer of hydrogen with antiparallel spins that form a singlet) based on the traditional parahydrogen induced polarization (PHIP) approach tackle this sensitivity problem with dramatic results. In recent years, the potential of this method for MRI has been recognized, and its impact on medical diagnosis is starting to be realized.



In this Account, we describe the use of *para*hydrogen to hyperpolarize a suitable substrate. This process normally involves the introduction of a molecule of *para*hydrogen into a target to create large population differences between nuclear spin states. The reaction of *para*hydrogen breaks the original magnetic symmetry and overcomes the selection rules that prevent both NMR observation and *para*hydrogen/*ortho*hydrogen interconversion, yielding access to the normally invisible hyperpolarization associated with parahydrogen. Therefore the NMR or MRI measurement delivers a marked increase in the detected signal strength over the normal Boltzmann-population derived result. Consequently, measurements can be made which would otherwise be impossible. This approach was pioneered by Weitekamp, Bargon, and Eisenberg, in the late 1980s. Since 1993, we have used this technique in York to study reaction mechanisms and to characterize normally invisible inorganic species. We also describe signal amplification by reversible exchange (SABRE), an alternative route to sensitize molecules without directly incorporating a molecule of *para*hydrogen. This approach widens the applicability of PHIP methods and the range of materials that can be hyperpolarized.

In this Account we describe our *para*hydrogen studies in York over the last 20 years and place them in a wider context. We describe the characterization of organometallic reaction intermediates including those involved in catalytic reactions, either with or without hydride ligands. The collection of *spectroscopic and kinetic data* with rapid inverse detection methods has proved to be particularly informative. We can see enhanced signals for the *organic products* of catalytic reactions that are linked directly to the catalytic intermediates that form them. This method can therefore prove unequivocally that a specific metal complex is involved in a catalytic cycle, thus pinpointing the true route to catalysis. Studies where a *pure nuclear spin state* is detected show that it is possible to detect all of the analyte molecules present in a sample using NMR. In addition, we describe methods that achieve the selective detection of these enhanced signals, when set against a strong NMR background such as that of water.

1. Account Overview

The principle of NMR is built around the interaction of magnetic nuclei with a magnetic field. Nuclei where the associated spin quantum number is 1/2, such as ^{1}H, ^{13}C, and ^{19}F, are readily studied by NMR because the lifetime of the associated nuclear spin states are generally sufficient to allow narrow line measurements to be made even though the associated NMR signal intensities are low and may need averaging. The importance of these signals is, however, reflected in their ability to provide diagnostic information

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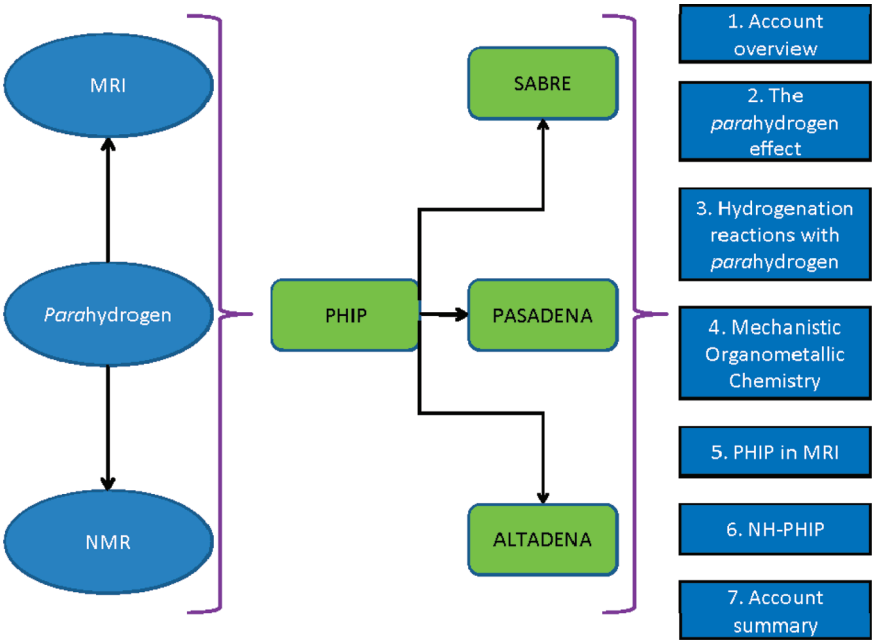

FIGURE 1. Applications of *para*-hydrogen derived hyperpolarization.

which leads to molecular characterization such as chemical shift, spin−spin coupling (*J*-coupling), and integral values. When coupled with two and higher dimensional approaches, even the structures of proteins have been revealed. Consequently, any method which increases the ability of NMR or MRI to detect materials is worth evaluation. In this context, *para*hydrogen derived magnetic polarization has the proven potential to increase this polarization level to unity, which corresponds to a signal enhancement of between 4 and 5 orders of magnitude over what is normally available. Since sensitivity relates to concentration, and scales with the square of the number of observations, measurement time and sample quantities can be dramatically reduced.

Although this Account focuses on dealing with polarization that originates in *para*hydrogen, we refer readers to contrasting brute force[1] and dynamic nuclear polarization (DNP)[2] methods for completeness. The relatively generic term *para*hydrogen induced polarization (PHIP) as used by Eisenberg to refer to this effect[3,4] encompasses the wide ranging nature of applications using this technology. However, Weitekamp coined the phrase *para*hydrogen and synthesis allow dynamic nuclear alignment (PASADENA)[5] to describe the resulting effect after hydrogenation in high magnetic field. Adiabatic longitudinal transport after dissociation engenders net alignment (ALTADENA)[6] similarly describes the results of low field hydrogenation. A further distinct approach, termed signal amplification by reversible exchange (SABRE), has also been described that polarizes products without the need for their hydrogenation.[7] A number of reviews report on the inorganic applications of PHIP[8−10] while the early review by Bargon and Natterer covers both low and high field transfer.[11]

2. Principles of the *Para*hydrogen Effect

In order to understand this effect, it must first be appreciated that molecular hydrogen actually exists as four nuclear spin isomers. The existence of these nuclear spin isomers derives from the symmetrization principle of quantum mechanics, which states that the overall wave function of the fermion dihydrogen must be antisymmetric with respect to exchange of nuclei. From this, it follows that antisymmetric rotational states in dihydrogen are associated with symmetric (*ortho*) nuclear spin states. Likewise, symmetric rotational states are associated with antisymmetric (*para*) nuclear spin states. *Ortho*hydrogen is used to describe hydrogen molecules that exist with one of the 3-fold degenerate spin state terms $\alpha\alpha$, $\alpha\beta+\beta\alpha$, and $\beta\beta$, whereas *para*hydrogen molecules exist solely as $\alpha\beta-\beta\alpha$ spin isomers. The *ortho* and *para* isomers exist in different rotational states, and their proportion is temperature dependent when their normally forbidden interconversion is facilitated.[12,13] If pure *para*hydrogen is desired, running hydrogen gas through a copper block containing silica/$FeCl_3$ catalyst at 20 K[9] is sufficient, and once formed and removed from the interconversion catalyst *para*hydrogen is relatively stable to spin-reequilibration. Hence, there is plenty of time for a chemical

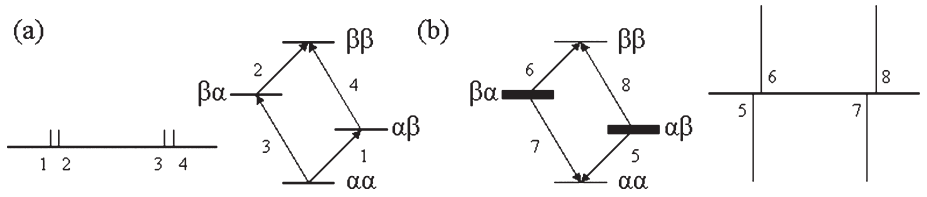

FIGURE 2. Energy levels and associated resonance profiles for ^{1}H NMR spectra produced for an AX type system with indicative Boltzmann-derived level populations (a) and *para*hydrogen-derived populations (b) represented by line thickness and transition labels 1−8.

reaction to take place that leads to reaction products which can be monitored by hyperpolarized NMR methods.

3. Hydrogenation Reactions with *Para*hydrogen

In 1986, Bowers and Weitekamp postulated that the incorporation of *para*hydrogen into a molecule would lead to a large product nuclear spin polarization.[14] They demonstrated this experimentally a year later when monitoring the hydrogenation of acrylonitrile by Wilkinson's catalyst.[5] Antiphase peaks of enhanced signal intensity were observed for both the propionitrile product, and the hydride signals of the metal catalyst in the corresponding ^{1}H NMR spectrum. Similar observations were also reported by the group of Eisenberg in the same year stemming from their hydrogenation studies of phenylacetylene by $Rh_2(CO)_2(dppm)_2(H)_2$.[15] A year later, Eisenberg placed these results in a *para*hydrogen context.[3]

Observations of this type are produced when a *para*hydrogen molecule is transferred into a reaction product in high magnetic field in such a way as to break its magnetic symmetry while retaining a spin−spin coupling between the two atoms (PASADENA). If two chemically distinct hydrogen atoms result, then the simple nuclear energy level distribution shown in Figure 2 readily explains this effect since if the *para*hydrogen spin state symmetry is conserved during reaction, only the $\alpha\beta$ and $\beta\alpha$ product states are populated (section 3c and Figure 4 provide further information related to this). The resulting population differences have been shown to approach unity, and the linked NMR spectra possess peaks of enhanced intensity with antiphase character that are separated by J_{HH}. The related ALTADENA situation occurs for reactions taking place in low field and typically lead to population of only one of either the $\alpha\beta$ or $\beta\alpha$ spin states.

3a. In Situ Photochemistry with *Para*hydrogen and Dihydride Complex Detection. Eisenberg and co-workers, completed the first detailed study of the addition of *para*hydrogen to a metal complex, in this case Ir(CO)(dppe)Br and Ir(CO)(dppe)(CN).[16] The detected hydride resonances for the corresponding H_2 addition products showed substantial signal enhancement in accordance with Figure 2. Related examinations using $IrCl(CO)(PPh_3)_2$ led to the observation of hydride resonances for well-known *cis*−*trans*-$Ir(CO)(PPh_3)_2Cl(H)_2$.[17] Subsequent investigations, at 298 K, established that a second hydride-containing product formed by addition over the P−Ir−P axis could be observed.[18] The signals for hydride ligands in this previously undetected minor product appear PHIP-enhanced, even though they are chemically equivalent, because they are magnetically second-order. These studies therefore demonstrated that PHIP not only leads to a signal enhancement in the hydride resonances of a dihydride product, but suggests that previously invisible species might be detected.

Nuclear spin polarizations of unity were actually shown to be generated in such metal dihydride products through a study involving the photochemical generation of [Ru(dpae)-$(CO)_2(H)_2$] (dpae =1,2-(diphenylarsino)ethane) from [Ru(dpae)-$(CO)_3$].[19] The two hydride ligands of [Ru(dpae)$(CO)_2(H)_2$] produce a detectable NMR signal that arises from a "transient" singlet state. This resulted in a 31 200-fold increase in the ^{1}H NMR signal strengths associated with the hydride ligand resonances of this species when observed at 9.4 T. This observation highlights the potential of PHIP to detect species that contain two hydrogen atoms that were previously located in the same *para*hydrogen molecule with dramatically improved sensitivity.

3b. The Role of Symmetry and Relaxation in the Detection of Metal Hydrides with PHIP. The key to observing such signal enhancements is that the process of H_2 addition is fast with respect to relaxation, and that the magnetic symmetry of the two formally dihydrogen nuclei is broken on moving to the product. Thus, when photochemically promoted H_2 addition to $Ru(CO)_2(dppe)$ was studied, substantial hydride signal enhancement was observed.[20] In contrast, the intermediate $Fe(CO)_2(dppe)$ produced no PHIP enhanced signals for the $Fe(CO)_2(dppe)(H)_2$ product due to its electronic triplet character.

Early studies of *para*hydrogen addition to $Rh(PMe_3)_4Cl$ and $Rh(PMe_3)_3Cl$ also serve to illustrate the importance of symmetry in detecting PHIP enhanced products.[21] In this case, both the $[Rh(PMe_3)_4(H)_2](Cl)$ and $Rh(PMe_3)_3(H)_2(Cl)$ products exhibit strongly enhanced hydride ligand signals albeit for different reasons; while the hydride ligands are magnetically inequivalent in both, only in the latter are they chemically different as revealed in Figure 3. Advanced NMR techniques, including COSY, HSQC, HMQC, and NOESY were also shown to enable the rapid probing of these PHIP enhanced species in this report.

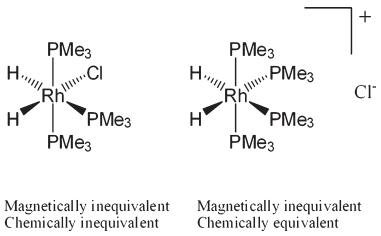

FIGURE 3. Chemical structures and magnetic properties of the hydride ligands of $Rh(PMe_3)_3Cl(H)_2$ and $[Rh(PMe_3)_4(H)_2]Cl$.

The ligand exchange reactions of stable 18-electron complexes such as $Ru(CO)_2L_2(H)_2$ (L = $AsMe_2Ph$, PMe_2Ph, PPh_3, and PMe_3) with *para*hydrogen have also been monitored.[22] Studies on $Ru(CO)_2(AsMe_2Ph)_2(H)_2$ revealed such species exist in *ccc*, *cct*-L, and *cct*-CO geometries[23] with their equilibrium ratios depending on L; only the *cct*-PMe_3 form is visible under normal conditions. Warming with *para*hydrogen enables the detection of elusive *ccc* isomers due to rapid free $H_2-Ru(H)_2$ exchange. In addition, the ^{13}CO isotopomer makes the major *cct*-L *para*hydrogen active by virtue of the now second order spin system associated with the square planar $M(CO)(^{13}CO)(H)_2$ core. Interestingly, not only are the ^1H hydride signals PHIP enhanced but so are those of the symmetry breaking ^{13}C and ^{31}P resonances.

3c. The Selective Observation of MR Signals Associated with PHIP. One of the key requirements when working with PHIP is to recognize the difference in the nuclear spin state description relative to that associated with thermally derived polarization. This effect is illustrated in Figure 4 which reveals how a normally observed hydride signal is in-phase while with *para*hydrogen an antiphase signal results.

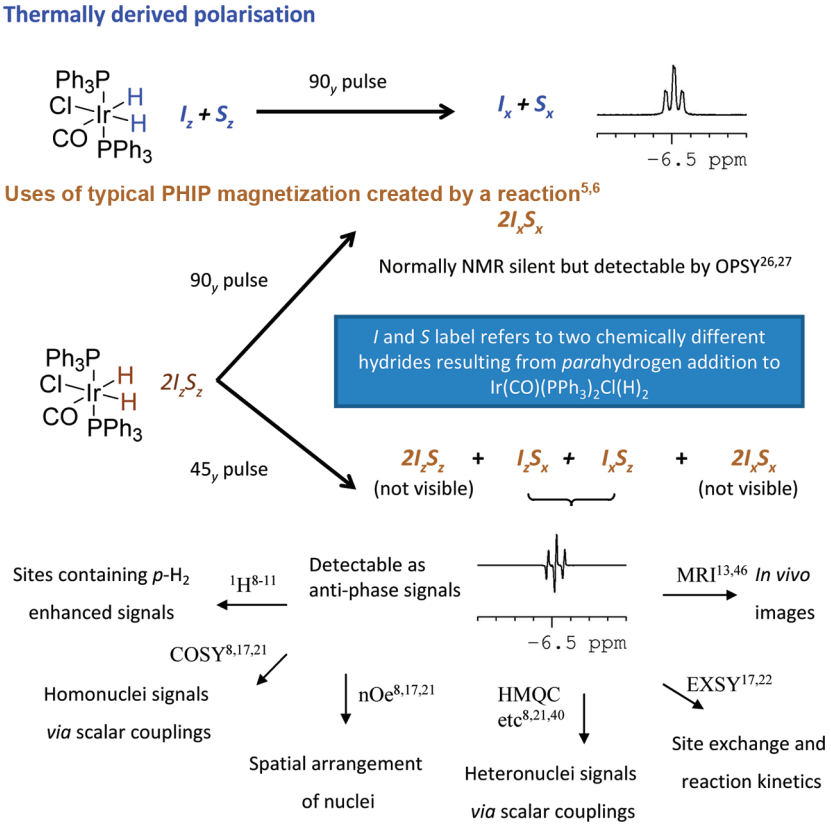

FIGURE 4. Magnetization generated under thermal and PHIP conditions; the associated signals seen for the low field hydride resonance of $Ir(CO)(PPh_3)_2Cl(H)_2$ are illustrated and key experiments referenced.

These differences have been used by Bargon to selectively observe protons previously located in a *para*hydrogen molecule by phase cycling,[24] while Aguilar et al. reported a more robust method called Only *Para*-hydrogen SpectroscopY (OPSY).[25] It uses pulsed field gradients to allow the selective observation of signals associated with *para*hydrogen derived spins or their coupling partners. This approach suppresses thermal signals associated with the substrate and solvent (Figure 5) and has been used two-dimensionally to probe coupled nuclei in such systems.[26] A further development makes use of the fact that metal centers bind many biologically relevant ligands to yield hydride signals in an uncongested region of the spectrum that are diagnostic of the resulting adduct. This approach enabled the detection of pyridine, purine, and adenine at picomole levels via binding to $Ir(PPh_3)_2Cl(H)_2$.[27] Furthermore, ^{15}N signals have been recorded for these low concentration adducts, and OPSY measurements made in protio solvents.

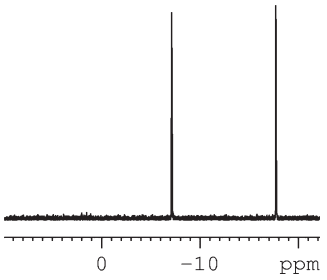

FIGURE 5. $^1H\{^{31}P\}$-OPSY NMR spectrum courtesy of Dr J. Agulia showing the result of monitoring PHIP enhanced $Ir(CO)(PPh_3)_2Cl(H)_2$ in protio tetrahydrofuran.

4. Mechanistic Organometallic Chemistry

It has now been shown that PHIP can be seen in a range of situations, as reflected in the following two subsections. These subsections illustrate studies related to hydrogenation, catalysis by clusters, and hydroformylation and demonstrate that metal dihydride, monohydride, and ancillary ligand signals such as those found in metal alkyl and vinyl groups can be detected through PHIP.

4a. PHIP Studies of Catalysis Involving Dihydride Complex Detection. The accepted alkene dihydride intermediate involved in hydrogenation by Wilkinson's catalyst, is *trans*-$Rh(alkene)(PPh_3)_2(Cl)(H)_2$. Surprisingly, when this reaction was monitored with *para*hydrogen and styrene, such an intermediate containing two *cis* phosphine ligands was detected. These solutions, however, also contain the dimeric complex $[Rh(\mu\text{-}Cl)(PPh_3)_2]_2$ (Figure 6).[28] Reaction of $[Rh(\mu\text{-}Cl)(PPh_3)_2]_2$ with *para*hydrogen confirmed earlier suggestions that $Rh(H)_2(PPh_3)_2(\mu\text{-}Cl)_2Rh(PPh_3)_2$ and the tetrahydride complex $[Rh(H)_2(PPh_3)_2(\mu\text{-}Cl)]_2$ can be readily formed. However, in the presence of an alkene and *para*hydrogen, signals corresponding to $Rh(H)_2(PPh_3)_2(\mu\text{-}Cl)_2Rh(PPh_3)$(alkene) were

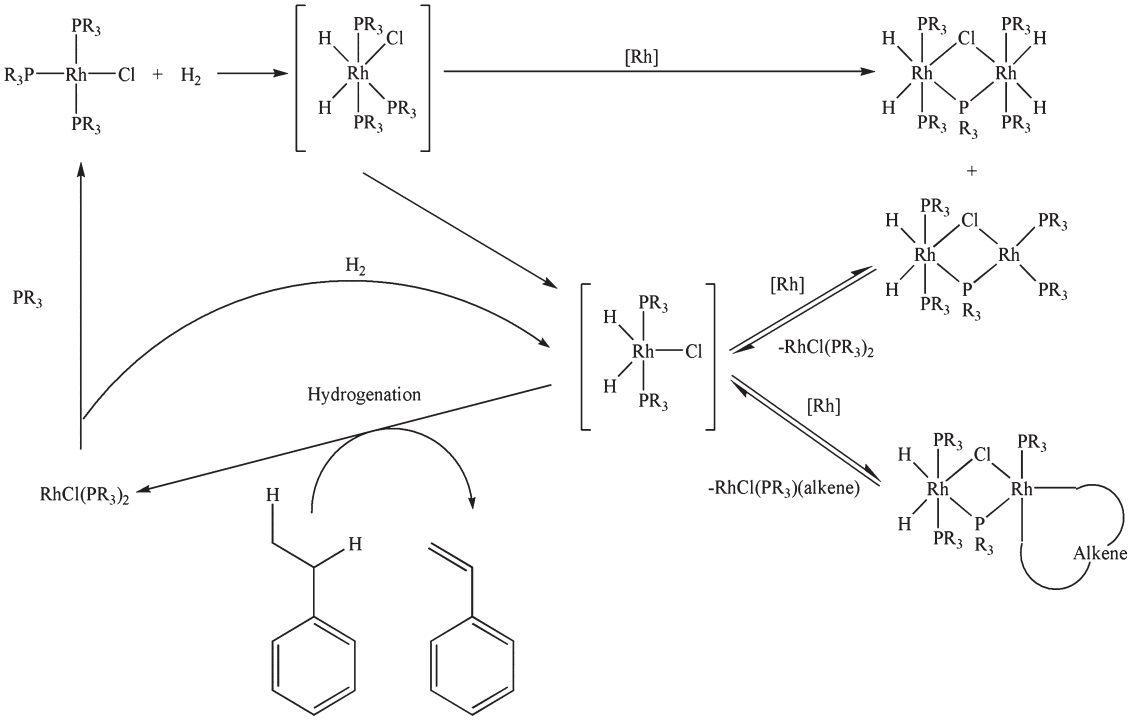

FIGURE 6. Species detected when the hydrogenation of an alkene by $[RhCl(PR_3)]_2$ is monitored using PHIP methods (R = Ph).

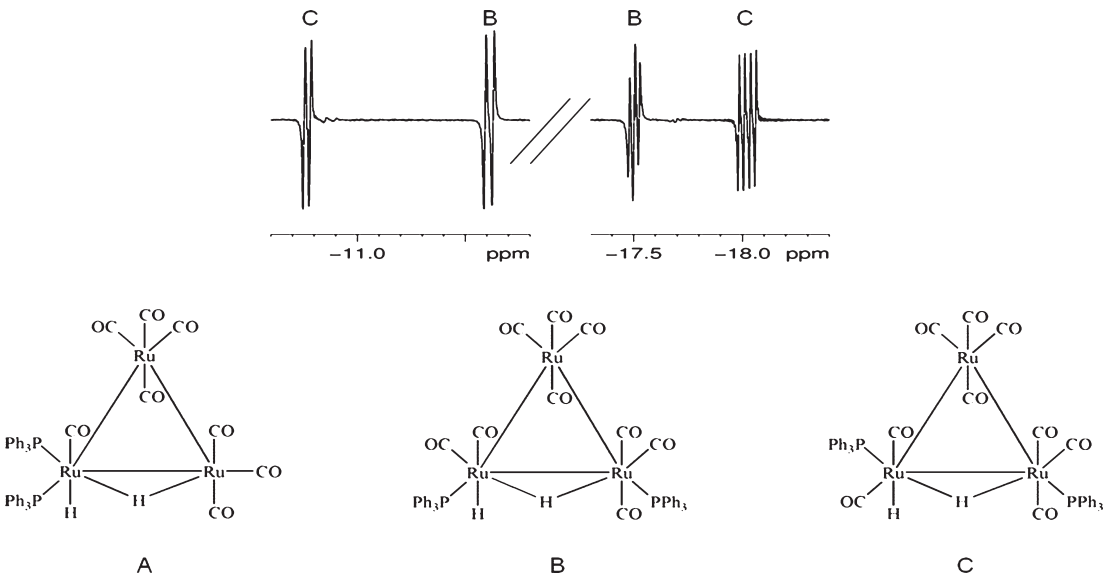

FIGURE 7. Proposed structures of the PHIP detected dihydride clusters formed by H_2 addition to $Ru_3(CO)_{10}(PPh_3)_2$; enhanced ^1H NMR resonances for terminal and bridging sites in B and C indicated above.

detected.[29] Direct hydrogen transfer from the hydride signals of $Rh(H)_2(PPh_3)_2(\mu\text{-}Cl)_2(Rh)(PPh_3)$(styrene) into ethylbenzene was observed from this previously unseen species with PHIP to proceed at a rate of 1 s^{-1} at 295 K. Studies on other binuclear dihydride complexes including $(H)(Cl)Rh(PMe_3)_2(\mu\text{-}H)(\mu\text{-}Cl)Rh(CO)(PMe_3)$, $(H)_2Rh(PMe_3)_2(\mu\text{-}Cl)_2Rh(CO)(PMe_3)$, and $HRh(PMe_3)_2(\mu\text{-}H)(\mu\text{-}Cl)_2Rh(CO)(PMe_3)$ revealed that such species play a similar role in hydrogenation catalysis. These studies therefore confirmed that when a metal center, possessing potential bridging ligands, is involved in catalysis, there is a real opportunity to produce binuclear resting states. Controlling access to them therefore provides a means to change reaction selectivity.

PHIP has also been used to investigate the structure, dynamics, and catalytic activity of clusters of the type $Ru_3(CO)_{12-x}(PPh_3)_x$ [where $x = 1-3$]. It was discovered that three isomers of $Ru_3(H)(\mu\text{-}H)(CO)_9(PR_3)_2$ exist (Figure 7),[30] and it plays a kinetically significant role in the hydrogenation of alkyne substrates according to EXSY methods.[31] A minor reaction pathway involving loss of phosphine yielded a product containing a pendant vinyl and a bridging hydride, with fragmentation forming less active mononuclear Ru(alkyne)$(CO)_2(PPh_3)(H)_2$. Significantly, these PHIP studies also revealed that polar solvents facilitate intact cluster catalysis while nonpolar solvents lead to increased cluster fragmentation (Figure 8).

These studies helped us recognize the importance the hydrogen cycling rate plays on the degree of signal amplification. When PHIP derived magnetization is encoded by an rf pulse, it relaxes back to a normal, thermally populated, magnetic state. Consequently, this method produces magnetization that can be read only once at high sensitivity. It is therefore the underlying chemical reaction that leads to the replenishment of high sensitivity magnetization. Thus, when the reaction is complete, new enhanced magnetization is only created if hydrogen loss occurs. One way to promote this process is to add a hydrogen acceptor to soak up the used metal hydride groups. This effect is illustrated in Figure 9 for hydrogenation by $RhI(CO)(PMe_3)_2$. The dramatic increase in signal strength is a result of the promotion of the rate of *para*hydrogen cycling through the system.

4b. PHIP within the Coordination Sphere of a Metal Complex. This Account outlines how metal dihydride complexes and their involvement in catalysis can be explored by PHIP. It is therefore possible to introduce *para*hydrogen into an organic material by hydrogenation. A number of reports where *para*hydrogen has provided insight into reaction mechanisms by linking these two approaches have also been reported.

One of these targeted a metal-η^3-allyl complex.[34] In this case, $Ir(\eta^3\text{-}C_3H_5)(CO)(PPh_3)_2$ was found to react with H_2 to form two isomers of $Ir(\eta^3\text{-}C_3H_5)(CO)(PPh_3)(H)_2$ after PPh_3 loss. Upon warming, *fac*- and *mer*-$Ir(CO)(PPh_3)_2(H)_3$ were formed in addition to propane and propene for which PHIP NMR signals were observed. This observation indicated reversibility in the final metal hydride transfer step. Upon reaction with a mixture of CO and H_2, these two hydrogenation products were suppressed and *cis*−*cis*-$Ir(CO)_2(PPh_3)(COCH_2CH{=}CH_2)(H)_2$

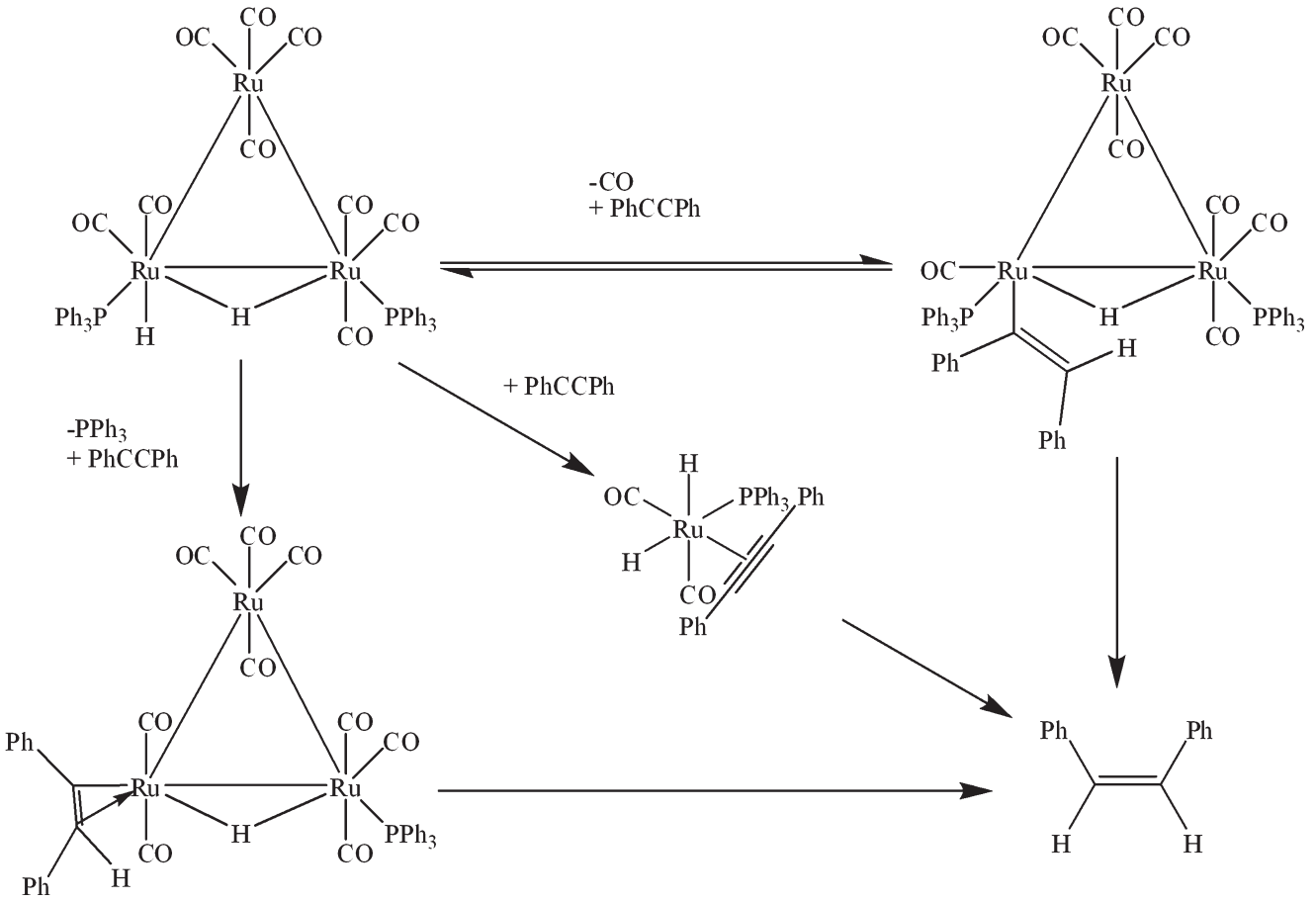

FIGURE 8. Reaction intermediates detected during the hydrogenation of diphenylacetylene by $Ru_3(CO)_{10}(PPh_3)_2$.[32]

and *cis*–*cis*-Ir(CO)$_2$(PPh$_3$)(η^1-CH$_2$CH=CH$_2$)(H)$_2$ were detected through their PHIP-enhanced hydride ligand signals. The latter species also showed PHIP in the η^1-CH$_2$ allyl resonance due to incorporation of a *para*hydrogen derived proton into this site, and spin–spin coupling to the partner hydride. This result therefore confirmed that an often elusive metal–alkyl-hydride can be detected by PHIP.

An analogous study of Co(η^3-C$_3$H$_5$)(CO)$_2$(PCy$_3$) catalyzed hydroformylation[35] led to Co(CO)$_3$(PCy$_3$)(COCH$_2$CH$_2$CH$_3$) and Co(CO)$_3$(PCy$_3$)(COCH(CH$_3$)$_2$) being detected through PHIP enhancements in the alkyl moiety resulting from hydrogenation of the allyl ligand. For PPh$_2$Me, additional signals due to Co(CO)$_2$(PPh$_2$Me)(propene)(COCH$_2$CH$_2$CH$_3$) and Co(CO)$_2$(PPh$_2$Me)(propene)(COCH(CH$_3$)$_2$) were detected. Furthermore, when the reactions of H$_2$ and diphenylacetylene were studied, the detection of Co(CO)$_3$(PPh$_2$Me)(CHPhCH$_2$Ph) rather than its acyl counterpart was achieved. Here, the PHIP enhanced signal profile of the PHIP enhanced products proved to provide information about the relative rates of key steps in the linear and branched hydroformylation products.[36] The PPh$_2$Me system proved to yield one of the largest differences in signal strength for the two aldehyde products, while producing Co(CO)$_3$(PPh$_2$Me)(COCH$_2$CH$_2$CH$_3$) and Co(CO)$_3$(PPh$_2$Me)(COCH(CH$_3$)$_2$) with similar PHIP signal intensities. These data therefore illustrated the existence of a complex link between turnover, concentration, and signal strength; H$_2$ addition to Co(CO)$_2$(PPh$_2$Me)(COCH$_2$CH$_2$CH$_3$) is more rapid than CO coordination. These data also revealed that the level of metal–alkyl/metal–alkene hydride equilibration and hence PHIP signal equilibration within the alkyl sites falls with both increase in temperature and productive aldehyde formation. Consequently, at low temperatures, reaction selectivity arises from the kinetic preference for hydride insertion to form a branched rather than linear intermediate. At higher temperatures, their rapid equilibration, evidenced by the randomly arranged *para*hydrogen label in the alkyl moiety, suggested that selectivity is controlled by thermodynamic stability, and the relative rates of H$_2$ addition to the corresponding 16-electron acyl intermediate.

One further example of such a reaction involves the hydrogenation of an alkyne. In this case, a traditional mechanism would take the alkyne, bind it to a metal

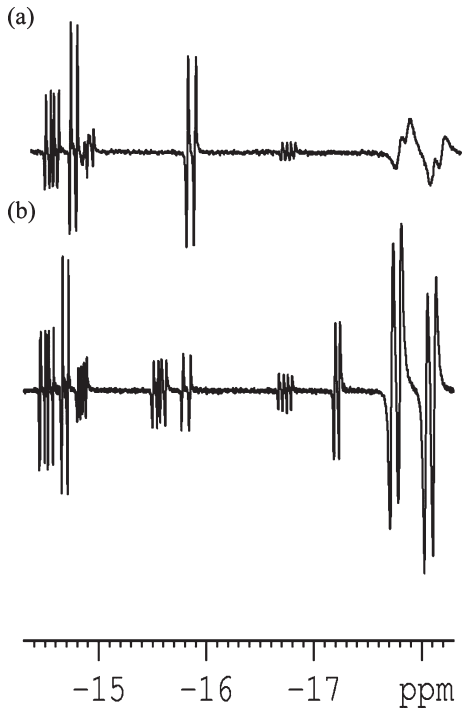

FIGURE 9. Hydride region of PHIP enhanced ^{1}H NMR spectra showing selected signals for products formed when $RhI(CO)(PMe_3)_2$ reacts with *para*hydrogen in the absence (a) and presence (b) of styrene.[33]

hydride, and form a metal vinyl hydride species which rearranges to the metal alkene prior to H_2 addition and production of a metal alkylhydride. These steps should be rapid, and might be reversible, but if a metal monohydride were to be involved, less reactive vinyl and alkyl species could be formed which might be detectable. A study of the hydrogenatation of alkynes, in methanol, using palladium *bis*-phosphine triflate catalysts provided such an example.[37] The use of PHIP and diphenylacetylene led to the detection of palladium *bis*-phosphine alkyl cation, where the alkyl ligands proton signals were sensitized. Rearrangement of this complex led to both *cis*- and *trans*-stilbene, via β-H transfer and a palladium monohydride cation. This hydride complex proved to react with diphenylacetylene to form a vinyl cation with both complexes showing very strong one-proton PHIP, an effect first observed by Eisenberg and Permin.[38] The characterization of the palladium monohydride and vinyl cations was aided by adding the co-ligand pyridine to reversibly block the vacant site in the *bis*-phosphine complexes $[P_2Pd]H^+$ and $[P_2Pd]CPh{=}CHPh^+$ thereby reducing catalytic activity by forming readily detectable adducts $[P_2Pd]H(py)^+$ and $[P_2Pd]CPh{=}CHPh(py)^+$. Collectively, these studies confirmed that PHIP can not only detect previously invisible reaction intermediates but also establish their kinetic significance in a wide range of chemical systems.

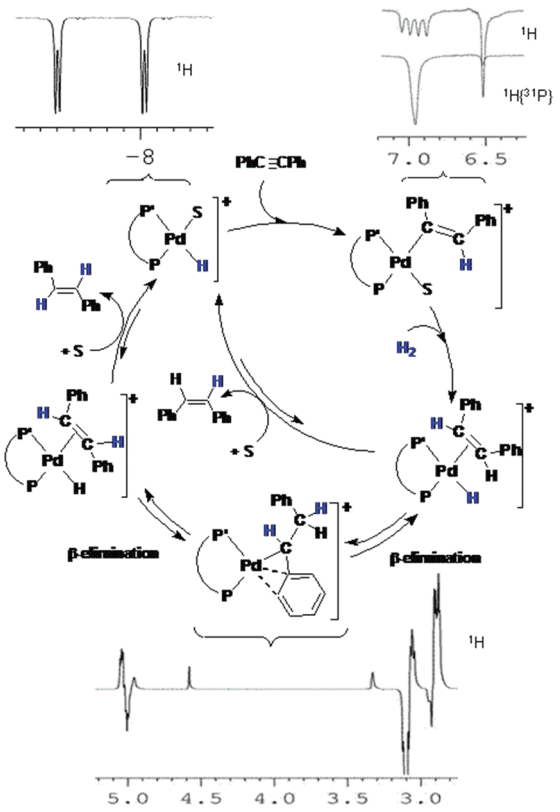

FIGURE 10. Mechanism of $Pd(P'{-}P)(OTf)_2$ ($P'{-}P$ is a chelated phosphine) catalyzed alkyne hydrogenation with inset ^{1}H NMR traces showing the PHIP signals seen for the indicated species.

These palladium containing species were characterized using inverse methods to detect key ^{31}P and ^{13}C signals in addition to COSY for confirming proton connectivity. However, they revealed one of the challenges of studying catalysis. Namely, for a 1% catalyst loading, with PHIP providing a 1000-fold signal enhancement, if 10% of the precatalyst exists at steady state in one of the potential intermediates, its signals are only comparable in intensity to those of the substrate. In other words, the dynamic range across the measured signals is still a potential problem. ^{2}H labeling overcomes this, which in the case of d_{10}-diphenylacetylene means that only product based *para*hydrogen derived protons can be detected. This approach has been used very successfully to improve intermediate detectability, and when extended to include ^{13}C labeling provides a viable route to high sensitivity studies of catalysis. These studies ultimately enabled the complex reaction pathway illustrated in Figure 10 to be confirmed.[39]

5. PHIP and Its Impact on Magnetic Resonance Imaging

In 1996, Bargon et al. recognized that, under ALTADENA conditions, transfer of proton magnetization to a slow

relaxing heteronucleus was possible if spin systems of the type ABX or A′AX′X were created.[40] Golman and co-workers utilized such a PHIP method to prepare and image samples of *para*hydrogenated acetylenedicarboxyldimethyl ester and maleic dimethyl ester.[41] The high signal strengths that result have allowed ^{13}C MRI angiograms to be recorded in less than 1 s. As a result, PHIP derived hyperpolarization is recognized as having the potential to impact on clinical MRI which rely on detecting a long-lived ^{13}C magnetic state rather than the more usual ^{1}H signal.[42] The creation and study of long-lived magnetic states is now a major area of NMR research led by Levitt and Carravetta, and Bodenhausen et al.[43,44]

It is also possible to use PASADENA to produce hyperpolarized samples for imaging. Roth et al. have reported on barbituric acids which display signal enhancements of >40 fold in the ^{1}H NMR spectrum.[45] 1-^{13}C-Succinate has also been hyperpolarized in conjunction with rf derived magnetization transfer and used to image brain cancer.[46] These approaches use equipment such as that reported by Hövener et al.[47] The ^{13}C labeled resonance of 1-^{13}C,2,3,3-D_3-hydroxyethyl acrylate (HEA) was hyperpolarized to a level of 18% in this case. Others have studied microreactors using ^{1}H-PHIP imaging with flow maps and activity profiles in a catalyst bed being resolved.[48] Recently, PHIP signals have also been observed in a heterogeneously catalyzed liquid-phase hydrogenation.[49] It is therefore clear that using PHIP to collect high sensitivity images for diagnosis, and the study of heterogeneous reactions marks one of the emerging areas for traditional PHIP over the next decade.

6. PHIP without *Para*hydrogen Incorporation into the Analyte

An alternative method to hyperpolarize a substrate using *para*hydrogen non-hydrogenatively has been developed. This method is described by the acronym SABRE (Signal Amplification By Reversible Exchange)[7] and avoids chemical modification of the substrate. Initially, a complex such as *fac*, *cis*-[Ir(PPh_3)(py)$_3$(H)$_2$]Cl was employed.[50] The pyridine ligands in this complex are labile, exchanging slowly into the bulk solution at a rate of 0.2 s^{-1} at 335 K. Hydride derived PHIP was transferred to ^{15}N nuclei by an INEPT based procedure, after which ligand exchange yields a free pyridine ^{15}N signal that was 120-times large than normal even though it does not contain any *para*hydrogen derived protons. A more dramatic effect has been observed when systems with weaker association between the metal and

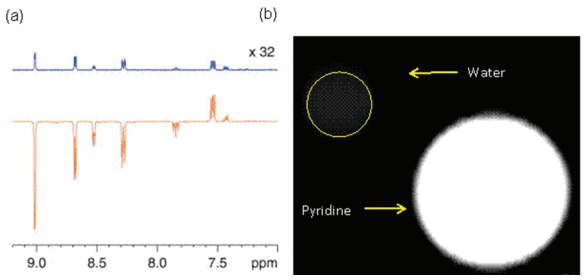

FIGURE 11. Traces illustrating >1000 signal enhancements derived from SABRE in conjunction with (a) the ^{1}H NMR detection of nicotinamide (normal trace top) and (b) the MRI detection of phantoms containing pyridine and H_2O.

substrate are studied in low magnetic field. In this case, transfer of nuclear spin polarization from *para*hydrogen to free pyridine molecules is observed without the need for radio frequency irradiation.[51] Signal enhancements in excess of 100-fold were observed for the ^{1}H, ^{13}C, and ^{15}N resonances of free pyridine with [Ir(PCy)$_3$(py)$_3$(H)$_2$]BF_4. The level of signal enhancement observed in the free substrate proved to increase as the metal center became simultaneously more electron rich and sterically encumbered. A true fast imaging measurement with steady state-precession (True-FISP), produced images showing a 160-fold signal enhancement.[7] Blümich and co-workers have recently shown using SABRE that nanomole amounts of pyridine can be detected;[52] a further report has demonstrated its use with peptides.[53] These approaches therefore polarize an analyte without the need for chemical incorporation of *para*hydrogen derived nuclei into it; all that is necessary is to bind reversibly to the metal center. Figure 11 demonstrates some of the potential benefits of SABRE.

A theoretical rational for this polarization transfer has also been published,[54] which explains how SABRE can be propagated through the scalar coupling network in the metal complex. Furthermore, the interplay between field dependent chemical shift and the field independent scalar coupling generates complex magnetic states that include potentially long-lived terms with both longitudinal two spin order and single spin longitudinal *z*-magnetization being readily observed (Figure 4). An apparatus to automate SABRE that facilitates signal averaging has also been described.[55] This study used the catalyst [Ir(IMes)(py)$_3$(H)$_2$]Cl to deliver >10 000-fold proton signal enhancement with pyridine.

7. Summary of PHIP Developments

This Account has illustrated the impact PHIP has made in the area of mechanistic inorganic chemistry. A number of

strategies to achieve PHIP within the coordination sphere of a metal have been described. While PHIP activity is readily achieved by the establishment of chemical inequivalence for the two *para*hydrogen derived protons, magnetic inequivalence engendered by labeling can also be used. Specific strategies include simple *para*hydrogen addition to form a metal dihydride, and more complex ones where unsaturated ligands are hydrogenated to form metal alkyl groups in situ. Additional methods where the semihydrogenation of an alkyne leads to a PHIP sensitized alkene allow secondary reaction products to be explored. Methods for the detection of PHIP magnetization including rapid 2D data acquisition and the OPSY protocol have been discussed which collectivity establish that *para*hydrogen provides detectable signals in a wide range of situations which even include the case where it is not formally incorporated into the analyte via SABRE. Here, no chemical modifications were required to a substrate even though a 10 000-fold increase in MR signal was demonstrated. The potential for PHIP in MRI has also been illustrated. Notwithstanding this, the scope of applications involving *para*hydrogen will undoubtedly continue to grow over the next decade.

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BIOGRAPHICAL INFORMATION

Simon Duckett was awarded a D.Phil. from the University of York in 1990 and undertook postdoctoral work with Prof. W. D. Jones and Prof. R. Eisenberg at the University of Rochester between 1990 and 1993. In 1993 he returned to the University of York to take up an appointment as Lecturer in Inorganic Chemistry and in 2004 was promoted to full Professor. He has authored over 60 publications in the *para*hydrogen field and has research interests in studying reactions mechanisms by NMR and MRI. He is currently director of the York Centre for Hyperpolarization in Magnetic Resonance which aims to see the *para*hydrogen effect utilized in the clinic.

Ryan Mewis obtained his Ph.D. from the University of Hull in 2009. He is currently a postdoctoral researcher with Professors Duckett and Green at the University of York where he is working on developing the SABRE approach.

FOOTNOTES

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The authors declare no competing financial interest.

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