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Hedegård, Erik D.; Sauer, Stephan P. A.; Milhøj, Birgitte O.

Published in:

Current Inorganic Chemistry

Publication date:

2013

Document version

Submitted manuscript

Citation for published version (APA):

Hedegård, E. D., Sauer, S. P. A., & Milhøj, B. O. (2013). On the use of Locally Dense Basis Sets in the Calculation of EPR Hyperfine Couplings: A study on Model Systems for Bio-inorganic Fe and Co complexes. *Current Inorganic Chemistry*, 3(3), 270-283.

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On the use of Locally Dense Basis Sets in the Calculation of EPR Hyperfine Couplings: A study on Model Systems for Bio-inorganic Fe and Co complexes

Birgitte O. Milhøj,^{*,†} Erik D. Hedegård,[‡] and Stephan P. A. Sauer^{*,†}

*Department of Chemistry, University of Copenhagen, DK-2100 Copenhagen Ø, Denmark,
and Department of Chemistry and Physics, University of Southern Denmark, Odense*

E-mail: birgitte.milhoj@chem.ku.dk; sauer@kiku.dk

The usage of locally dense basis sets in the calculation of Electron Paramagnetic Resonance (EPR) hyperfine coupling constants is investigated at the level of Density Functional Theory (DFT) for two model systems of biologically important transition metal complexes: One for the active site in the compound 0 intermediate of cytochrome P450_{cam}, $[\text{Fe}(\text{OOH})(\text{SH})(\text{en})_2]^+$, and one for the active site in coenzyme B₁₂, $[\text{Co}(\text{NH}_3)(\text{CN})(\text{en})_2]^+$. The Fermi contact, spin-dipolar and second order paramagnetic spin-orbit coupling contributions to the hyperfine coupling tensors of the metal and the ligating ethylenediamine N atoms are calculated, and their dependence on the basis set for the remaining atoms are investigated. Core property basis sets are employed for the metals (aug-cc-pVTZ-Juc) and their equatorially coordinating N atoms (aug-cc-pVTZ-J or 6-31G-Juc analogues to the Pople style basis sets used for the remaining atoms), while smaller correlation-consistent or

*To whom correspondence should be addressed

[†]University of Copenhagen

[‡]University of Southern Denmark

Pople style basis sets are used for the remaining, so-called “non-coupled”, atoms. Most of the investigated basis set combinations are found to give results which differ by less than 1% from the results obtained with core property basis sets on all atoms. We find thus for the cytochrome model system that using the small 6-31G(d) basis set on the non-coupled atoms together with core property basis sets on the Fe and N atoms gives essentially converged results. It is found to be mostly the second order paramagnetic spin-orbit interaction that demands the use of larger basis sets on the non-coupled atoms. If, however, an error of less than 0.5 MHz is sufficient any basis set can be used for the non-coupled atoms. For the cobalt containing model system the 6-31G(2d) basis set generally gives results within 1% of the reference value.

1 Introduction

Intermediates in the catalytic cycles of metalloproteins often have unpaired electrons and can therefore potentially be studied by EPR spectroscopy. Some of the most prominent examples are the cytochrome P450 monooxygenases which catalyse hydroxylation of inactive C-H bonds in many different vital reactions *e.g.* regulation of the metabolism of pharmaceutical substances as well as biosyntheses of metabolites, steroid hormones and anti-hypertensive substances.¹ The active site in cytochrome P450 proteins contains an iron atom, hexacoordinated by a protoporphyrin ring, a cysteine from the protein backbone and a sixth, axial, ligand which in the resting state is typically a water molecule. The most intensively studied member of this family is probably cytochrome P450_{cam} which catalyses the hydroxylation of camphor to 5-exo-hydroxycamphor. Detailed knowledge of the reaction mechanism in the catalytic cycle, during which the oxidation state of iron changes from II to IV, is a prerequisite for realising the great potential in mimicking this reactivity in designer enzymes.

Even though cytochrome P450_{cam} has been investigated by x-ray crystallography, the structures of some of the intermediates of the catalytic cycle are not completely known.²

Theoretical studies have so far mostly based their conclusions concerning the active site of enzymes on calculated reaction and activation energies.³⁻⁷ On the other hand, since Davydov *et al.* have measured EPR spectra for several of the intermediates,⁸⁻¹² one might as well support or contradict the conclusions of energy based studies by comparing the measured hyperfine constants with calculated constants for the proposed geometrical and electronic structures of these intermediates. Both the metal hyperfine and also the ligand super hyperfine couplings are interesting in this aspect.

However, it is by now well known,^{13,14} that for properties, which involve the interaction between electronic and nuclear spins, the electron density close to the nucleus has to be described much better than it is possible with standard energy-optimized, one-electron basis sets consisting of Gaussian functions.¹⁵⁻³⁵ Several series of specialised core-property basis sets have therefore been developed for the main group elements over the years^{23,24,32,35-44} but only few for transition metals.^{28,45,46} One of them, the aug-cc-pVTZ-J series of basis sets,^{24,36-41,45} is the only one which has basis sets for the d-block metals Sc-Zn⁴⁵ in addition to the main group atoms. Employing these basis sets enables the calculation of coupling constants in good agreement with experimental values,^{45,47,48} but these basis sets are also significantly larger than the basis sets typically employed in calculations on gas-phase or QM/MM models for the active site of cytochrome P450 enzymes,^{4,7} which hampers their application. A way out of this dilemma is to employ a locally dense basis set approach in which core-property basis sets are used only on the atoms of interest along with smaller standard basis sets for the remaining atoms. This approach has previously been successfully employed in the calculation of Nuclear Magnetic Resonance (NMR) shielding and coupling constants.⁴⁹⁻⁵⁴

In this study we investigate, therefore, whether the locally dense basis set methodology can also be employed in DFT calculations of EPR hyperfine coupling constants. To this purpose we have created a simple model, $[\text{Fe(III)(OOH)(SH)(en)}_2]^+$, of the compound 0 intermediate of the cytochrome P450_{cam} catalytic cycle, where the heme ring in the iron-

protoporphyrin complex is modelled by two ethylenediamine groups and the cysteine connection to the rest of the protein by a thiol group as shown in Figure 1a. For this model, which is called **Ia** in the following, we have extensively studied the dependence of the calculated hyperfine coupling constants of Fe and the equatorially coordinating N ligand atoms on the basis sets on the other, so-called non-coupled, atoms in the complex.

Another important metal containing biomolecules is coenzyme B₁₂. The Co atom in coenzyme B₁₂ is coordinated by a corrin ring, nitrogen in a histidine and a weakly bound carbon in either a methyl or a cyano group. This metal-carbon bond is unusual in enzymes and it is believed that this bond is the reason for the reactivity of coenzyme B₁₂.⁵⁵ In its normal form coenzyme B₁₂ is EPR silent, but coenzyme B₁₂ radicals are found to be created in the catalytic cycle of coenzyme B₁₂^{56,57} or in ethanolamine deaminase.⁵⁸⁻⁶⁴ As a second test system we have therefore carried out the basis set study also on the Co-complex [Co(II)(NH₃)(CN)(en)₂]⁺, called model **IIa** in the following. This complex is shown in Figure 1b, and is meant as a model system for coenzyme B₁₂.

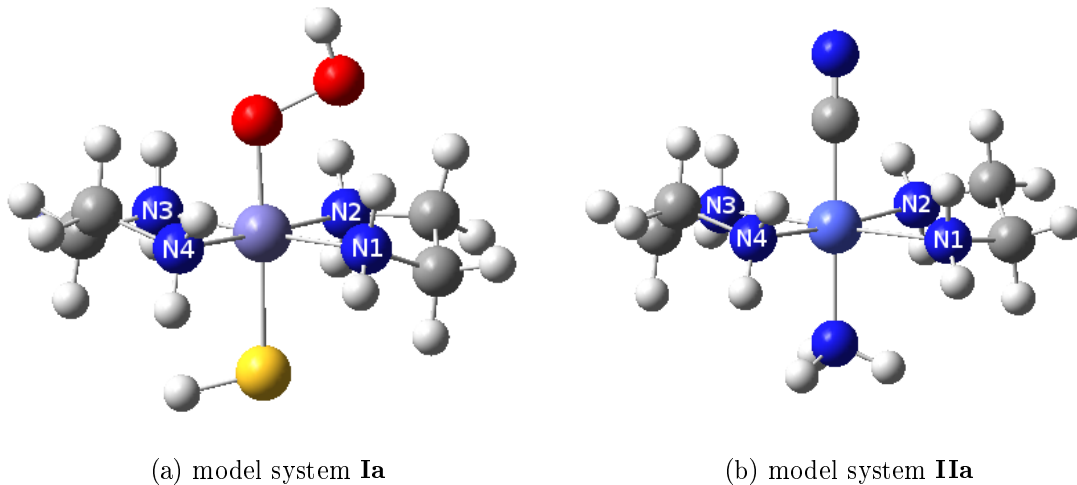


Figure 1: Structures of model systems **Ia** and **IIa** optimised at the B3LYP/aug-cc-pVDZ level.

In the following we will describe the details of our computational studies and then discuss the results of the locally dense basis set study for the iron and cobalt complexes. Finally

we will present calculated hyperfine and super-hyperfine tensors for two significantly larger model system **Ib** and **IIb**, as typically employed in gas phase studies⁴ of the active site of the compound 0 intermediate of cytochrome P450_{cam} and for the EPR active intermediate of coenzyme B₁₂ cyanocobalamin complex.

2 Computational Details

The geometry of the two model systems for the locally dense basis set study, **Ia** and **IIa**, both in a doublet spin state, were optimised at the B3LYP/aug-cc-pVDZ level, while the two larger model systems, **Ib** and **IIb**, were optimised at the B3LYP/6-311G(d). All optimizations were carried out with the Gaussian09⁶⁵ programme suite. The resulting structures are shown in Figures 1 and 2. Using the ORCA⁶⁶ programme suite the isotropic Fermi contact (A^{FC}), the anisotropic spin-dipolar (A^{SD}) and the second order paramagnetic spin-orbit (A^{PSO}) coupling contributions were calculated for the hyperfine coupling tensors of iron and cobalt as well as for the super-hyperfine coupling tensors of the equatorially coordinating nitrogen atoms of the ethylenediamine ligands (henceforth named N1–N4 as seen in Figure 1). For the latter nitrogen super-hyperfine coupling tensors, both the A^{SD} and A^{PSO} terms are negligible, and will only be reported in the Supporting Material. Detailed expressions for the A^{FC} and A^{SD} contributions can be found in advanced textbooks.¹³ The A^{PSO} contribution can be evaluated by several procedures^{67–72} and is here calculated as described in ref. 67. The two electron contribution to the A^{PSO} contribution is computationally difficult to handle and is often, as done by ORCA, replaced by an effective two electron operator based on effective potential and mean-field approaches.

In addition to the spin-orbit coupling, which gives rise to the A^{PSO} contribution, also scalar relativistic^{70–74} and finite nuclear volume effects^{70–72,74,75} are known to effect hyperfine coupling constants. While the latter are generally small for first row transition metals as studied here, scalar relativistic effects could play a role. Scalar relativistic and spin-orbit ef-

fects are obviously included in four-component calculations of hyperfine coupling constants,⁷⁵ but scalar relativistic effects can also be included via various two-component approaches such as the Douglas-Kroll-Hess,⁷⁴ the zeroth-order regular approximation⁷⁰⁻⁷² or the normalised elimination of the small component approaches⁷³ to name a few. As the scalar relativistic effects do not give rise to an additional contribution to the hyperfine couplings but only modify the three contributions studied here and as their contribution is not expected to be influenced by the choice of basis set on the non-coupled atoms, we have not considered them in this work.

For the metal atoms, the aug-cc-pVTZ-J basis sets⁴⁵ in their uncontracted form, henceforth referred to as aug-cc-pVTZ-Juc, were employed, whereas for the four nitrogen atoms of the ethylenediamine ligands the contracted version³⁸ was used. These basis sets are derived from the correlation consistent aug-cc-pVTZ basis sets⁷⁶⁻⁷⁹ by adding tight s-, p- and d-type Gaussian functions until saturation of either the NMR spin-spin or the EPR hyperfine coupling constants, removal of the second polarisation function with the smallest exponent and recontraction with molecular orbital coefficients. They consist then of (13s,3p,1d) primitive Gaussian functions contracted to [6s,3p,1d] for hydrogen, (15s6p3d1f) contracted to [9s5p3d1f] for second row atoms and (25s,18p,10d,3f,2g) contracted to [17s,10p,7d,3f,2g] for first row transition metals as compared to the (6s,3p,2d) contracted to [4s,3p,2d], (11s,6p,3d,2f) contracted to [5s,4p,3d,2f] and (21s,17p,9d,3f,2g) contracted to [8s,7p,5d,3f,2g] in the original aug-cc-pVTZ basis sets. As a smaller alternative also the 6-31G-J basis sets⁴³ augmented with diffuse and polarisation functions were employed for the nitrogen atoms in model system **Ia**. These basis sets were applied in their uncontracted form (where they are still smaller than the contracted aug-cc-pVTZ-J basis sets). The 6-31G-J basis sets were generated in almost complete analogy to the aug-cc-pVTZ-J basis sets from Pople's 6-31G basis sets⁸⁰ with the only difference that the contraction coefficients were taken from Hartree-Fock calculations on the atoms and not molecules as was the case for the aug-cc-pVTZ-J basis sets. They consist then of (13s,5p) primitive Gaussian functions contracted to [6s,2p] for nitrogen

as compared to the (10s,4p) contracted to [3s,2p] in the original 6-31G basis set. For the other atoms, which will be called "non-coupled" in the following, a broad selection of the standard correlation consistent^{76-78,81} or Pople style^{80,82-85} basis sets, given in Table 1, were used. Whenever the 6-31G-Juc basis set is used on the N atoms, the same polarisation functions are added as are added to the Pople basis on non-coupled atoms. Table 1 also shows the total number of contracted basis functions for model system **Ia** (model system **IIa** has a similar amount of functions) as well as the abbreviations used for shorter notation in the following for the individual basis set combinations. As reference for the calculations with the locally dense basis sets, we use the calculation with the aug-cc-pVTZ-Juc basis set on the metal atoms and the aug-cc-pVTZ-J basis set on all other atoms.

All calculations discussed here have been carried out with the B3LYP^{86,87} exchange-correlation functional, however in the Supporting Material we include also results obtained with the PBE,^{88,89} and PBE0⁹⁰⁻⁹² functionals, which show that the basis set dependence is analogous and the conclusions are the same for these two functionals as for B3LYP.

3 Locally Dense Basis Set Study

In order to base our conclusions not only on the $[\text{Fe}(\text{OOH})(\text{SH})(\text{en})_2]^+$ complex (**Ia**) with Fe in a formal d^5 electron configuration, we have carried out part of the study also on model system **IIa**, which is the $[\text{Co}(\text{NH}_3)(\text{CN})(\text{en})_2]^+$ complex with cobalt in a formal d^7 configuration. Despite a similar ligand sphere the individual contributions to the hyperfine couplings are quite different for the Co-complex and the Fe-complex. For the reference basis set we find that in absolute values all the contributions are between 3.5 and 19 times larger in model system **IIa** than in **Ia**. Furthermore, the importance of the three contributions, the Fermi contact (A^{FC}), spin-dipolar (A^{SD}) and second order paramagnetic spin-orbit (A^{PSO}), are quite different as well. Whereas in model system **Ia** the largest A^{SD} diagonal element is only about twice as large as the A^{FC} contribution and the isotropic value of the A^{PSO}

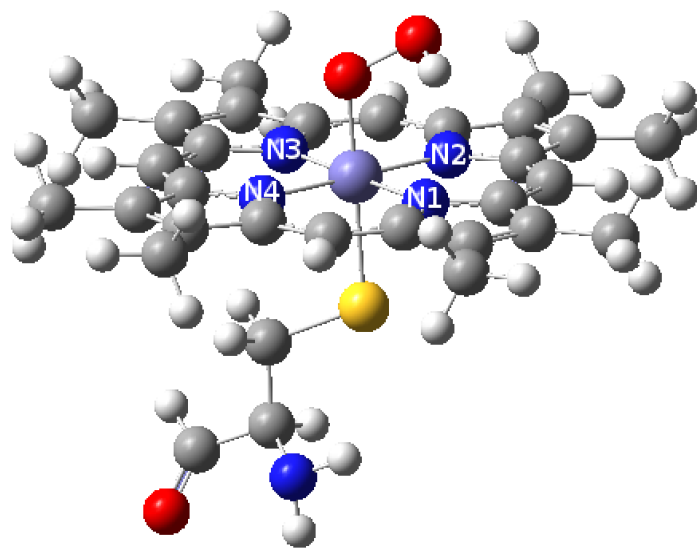
Table 1: Basis sets employed for the non-coupled atoms in combination with either the aug-cc-pVTZ-J or 6-31G-Juc^(a) basis sets on the N1–N4 in Figure 1. Also the total number of basis functions are given for model **Ia** with both aug-cc-pVTZ-J and 6-31G-Juc on N (model system **IIa** has a similar number)

Basis set on “rest”	Abbreviations		# cont. func.	
	aug-cc-pVTZ-J	6-31G-Juc ^(a)	aug-cc-pVTZ-J	6-31G-Juc ^(a)
6-31G	Pdz	P’dz	455	383
6-31+G	Pdz+	P’dz+	483	427
6-31++G	Pdz++	P’dz++	501	445
6-31G(d)	Pdz1*	P’dz1*	490	438
6-31G(2d)	Pdz2*	P’dz2*	525	493
6-31G(2df)	Pdz3*	P’dz3*	574	570
6-31G(d,p)	Pdz1**	P’dz1**	544	492
6-31G(2d,2p)	Pdz2**	P’dz2**	633	601
6-31G(2df,2pd)	Pdz3**	P’dz3**	772	768
6-311G	Ptz		505	—
6-311+G	Ptz+		533	—
6-311++G	Ptz++		551	—
6-311G(d)	Ptz1*		540	—
6-311G(2d)	Ptz2*		575	—
6-311G(2df)	Ptz3*		624	—
6-311G(3df)	Ptz4*		659	—
6-311G(d,p)	Ptz1**		594	—
6-311G(2d,2p)	Ptz2**		683	—
6-311G(2df,2pd)	Ptz3**		822	—
cc-pVDZ	Ddz		544	—
cc-pVTZ	Dtz		818	—
aug-cc-pVDZ	aDdz		679	—
aug-cc-pVTZ	aDtz		1092	—
aug-cc-pVTZ-J	reference		1051	—

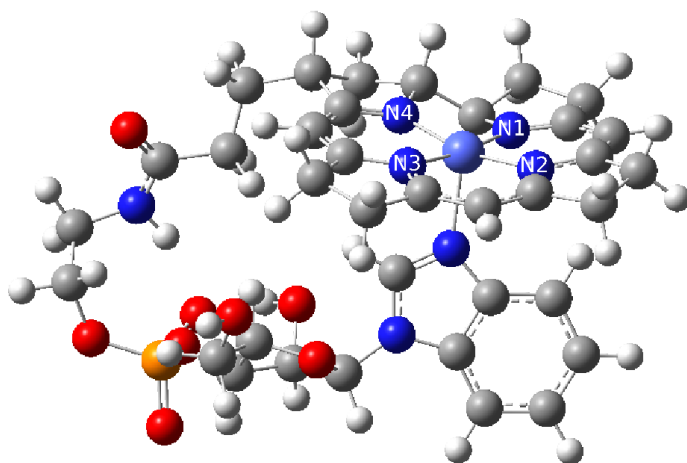
^(a) where the same polarisation functions are added to the 6-31G-Juc basis set on the N atoms as are added to the non-coupled atoms

corrections is about one fourth of the A^{FC} term, we find for model system **IIa** that the largest A^{SD} element is more than six times larger than the A^{FC} term and even the A^{PSO} term is larger. Finally, the super-hyperfine couplings to the nitrogen atoms differ strongly from the ones in model system **Ia**. Model system **IIa**, although structurally quite similar, is thus nevertheless a sufficiently different second test case. The various coupling contributions

to the hyperfine couplings of the metal atoms and to the super-hyperfine couplings of the nitrogen atoms of the ethylenediamine ligands are discussed separately (deviations of the A^{SD} and A^{PSO} contributions for the nitrogens are negligible and are therefore not discussed). In the figures the reference values are shown as black dashed horizontal lines.



(a) model system **Ib**



(b) model system **IIb**

Figure 2: Structures of model systems **Ib** and **IIb** optimised at the B3LYP/6-311G(d) level.

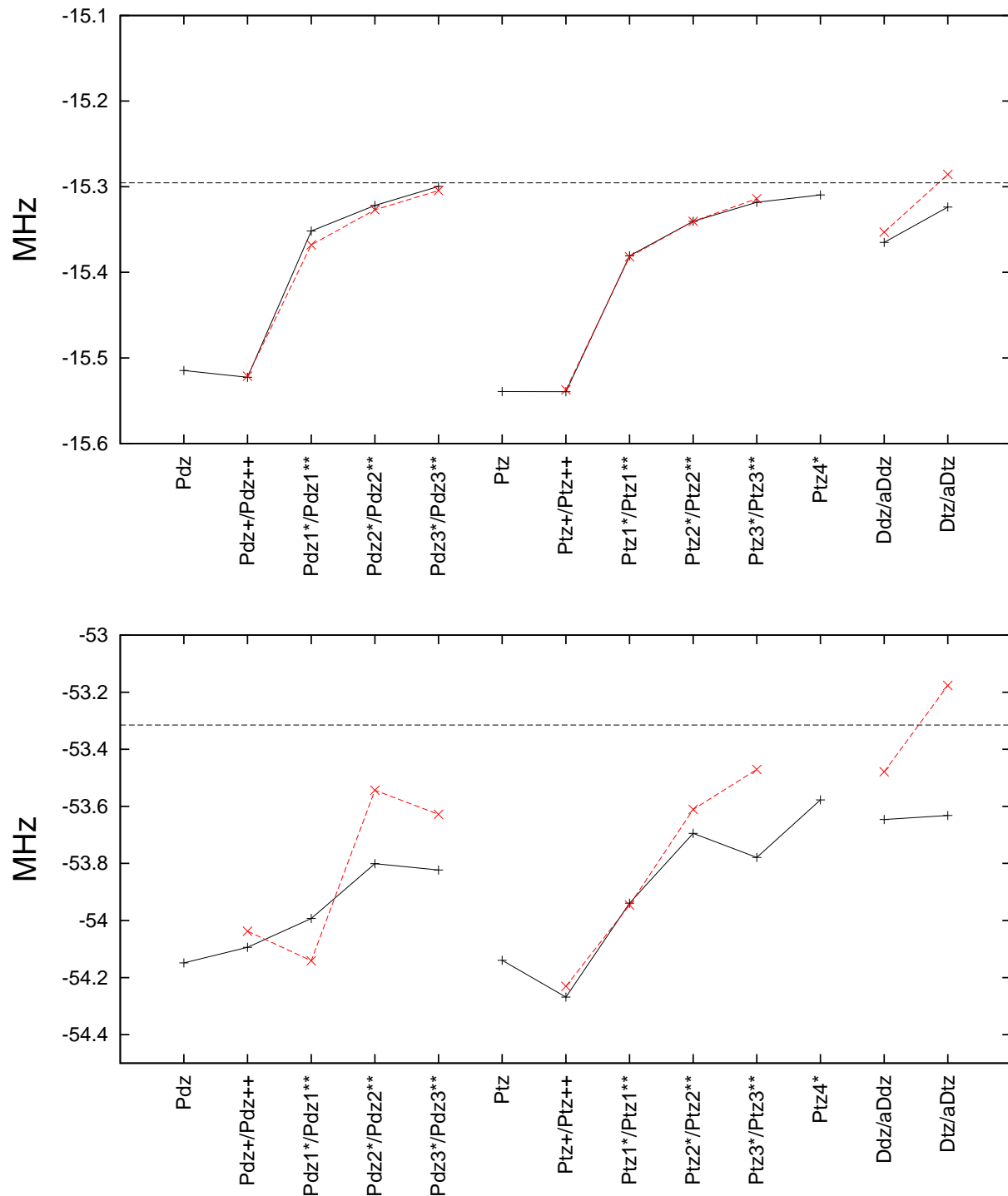


Figure 3: Calculated A^{FC} term for Fe (top) and Co (bottom) versus the basis set on the non-coupled atoms. The basis set on atoms N1–N4 (*cf.* Figure 1) is aug-cc-pVTZ-J. The (+) symbols indicate that diffuse or polarization functions are not used on hydrogen, while (x) includes additional diffuse and/or polarisation functions on hydrogens. The dashed horizontal line indicates the reference calculation. For details on the labels see Table 1.

3.1 Hyperfine coupling of the metal atoms

3.1.1 Fermi contact contribution

Using the aug-cc-pVTZ-J basis set on N1–N4: In Figure 3 (top) the influence of the choice of basis set on the non-coupled atoms is shown for the Fermi contact contribution to the hyperfine coupling of iron in model system **Ia** (see also Table S1 in the Supporting Material). The reference value is $A^{\text{FC}}(\text{Fe}) = -15.30$ MHz. Using smaller basis sets on the non-coupled atoms gives more negative values for $A^{\text{FC}}(\text{Fe})$. Only with the aug-cc-pVTZ basis set on these atoms does the Fermi contact contribution become slightly larger than the reference value due to the additional diffuse f-function in the aug-cc-pVTZ basis set. It is seen that changing the basis for the non-coupled atoms from aug-cc-pVTZ-J (the black dashed line) to any of the other correlation consistent basis sets or a Pople style basis set with at least one polarisation function on the non-hydrogen atoms increases the absolute value of the $A^{\text{FC}}(\text{Fe})$ term by less than 0.1 MHz or 0.7%. Deviations of less than 1%, for the Fermi contact contribution with respect to the reference, can therefore be obtained with a small basis set with a single added polarisation function on the non-hydrogen atoms. On the other hand, if deviations of about 0.5 MHz (which is still a small error) are sufficient then even a 6-31G basis set on non-coupled atoms will be adequate (as can also be seen from the Supporting Material).

Turning to the Co hyperfine couplings in model system **IIa** we see from Figure 3, or Table S2 in the Supporting Material, that both the absolute value and the variation of the Fermi contact term with the choice of basis sets are larger than for Fe in complex **Ia**. The reference value obtained is $A^{\text{FC}}(\text{Co}) = -53.32$ MHz. Using smaller basis sets on the non-coupled atoms gives again more negative values for $A^{\text{FC}}(\text{Co})$ with the exception of the aug-cc-pVTZ basis set. It is seen that changing the basis for the non-coupled atoms from aug-cc-pVTZ-J to any of the other correlation consistent basis sets changes the value of the $A^{\text{FC}}(\text{Co})$ term by less than 1% or 0.5 MHz. For the Pople type basis sets on the non-coupled atoms there is again

no important difference between employing valence double- or valence triple-zeta basis sets. Important is the addition of polarisation functions, however, and contrary to the Fe-complex one has to add two first polarisation functions on the non-hydrogen atoms, *i.e.* 2 d-type functions like in the 6-31G(2d) basis set, in order to force the deviation from the reference value below 1% which corresponds to 0.5 MHz. Furthermore, it can be seen that adding the first f-type function increases the deviation but that the second d-type function makes up for this. Also the extra functions on the hydrogens make a slightly more noticeable difference than for the Fe-complex, but they are not necessary in a basis set with two polarisation functions as the deviation is already below 1% (or 0.5 MHz) without including them.

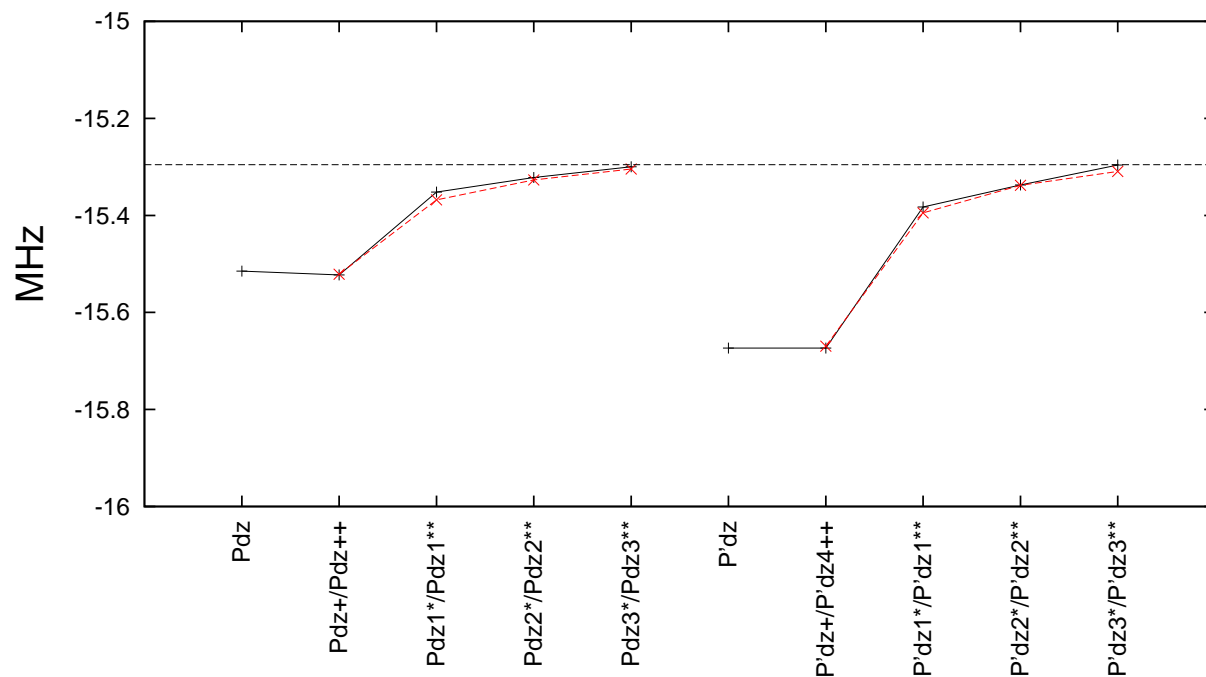


Figure 4: Calculated $A^{\text{FC}}(\text{Fe})$ with various diffuse and polarisation functions added to the 6-31G-Juc basis set on N1–N4 (see Figure 1) and to the 6-31G basis set on the non-coupled atoms. For details of the labels see Table 1. The results for aug-cc-pVTZ-J on the N atoms are shown on the left for comparison. The (+) symbols indicate that diffuse or polarization functions are not used on hydrogen, while (x) includes additional diffuse and/or polarisation functions on hydrogens.

Using the 6-31G-Juc basis set on N1–N4: Also the totally uncontracted "-Juc" version of the Pople style basis sets, developed by Kjær *et al.*,⁴³ has been employed on the nitrogen atoms of the ligands in combination with normal Pople style basis sets on the other atoms of the ligands in model system **Ia**. From Figure 4, and the results in Table S3 in the Supporting Material, it can be seen that this gives slightly more negative values for the $A^{\text{FC}}(\text{Fe})$ term than using the aug-cc-pVTZ-J basis sets on the nitrogens. However, the changes are at most 0.15 MHz or 1% of the reference value. Using a 6-31G(d)-Juc on N1–N4 and 6-31G(d) basis sets on non-coupled atoms gives thus a deviation of 0.1 MHz or 0.7% from the reference and adding more polarisation functions reduces this difference even further. This implies that, as far as the Fermi contact contribution to the hyperfine coupling of Fe is concerned, the 6-31G(d)-Juc basis set can be used on the coordinating N atoms as a smaller, and thereby computationally faster, alternative to the aug-cc-pVTZ-J basis set. Again, polarisation functions on the hydrogens do not significantly change the results, while adding only diffuse functions on the non-coupled atoms is not sufficient.

3.1.2 Anisotropic spin-dipolar contributions

Using the aug-cc-pVTZ-J basis set on N1–N4: In model system **Ia** the $A_{11}^{\text{SD}}(\text{Fe})$ contribution to the hyperfine coupling of Fe obtained with the reference basis is 14.97 MHz, which is of the same order of magnitude as the isotropic FC term but the smallest of the three spin-dipolar tensor diagonal elements in absolute terms. As it can be seen from Table S4 in the Supporting Material it exhibits no real variation with the choice of basis set on the non-coupled atoms. The value of the $A_{22}^{\text{SD}}(\text{Fe})$ contribution is with the reference basis set 21.28 MHz and for the $A_{33}^{\text{SD}}(\text{Fe})$ contribution, finally, holds that $A_{33}^{\text{SD}}(\text{Fe}) = - (A_{11}^{\text{SD}}(\text{Fe}) + A_{22}^{\text{SD}}(\text{Fe}))$. The variation with the basis set is thus almost equal for the $A_{33}^{\text{SD}}(\text{Fe})$ and $A_{22}^{\text{SD}}(\text{Fe})$ contributions and we discuss only the results for the latter in Figure 5 and Table S5 in the Supporting Material. Contrary to the $A^{\text{FC}}(\text{Fe})$ contribution, the deviation from the reference is only consistently below a 0.1 MHz threshold if second polarisation functions, *i.e.* f-type functions,

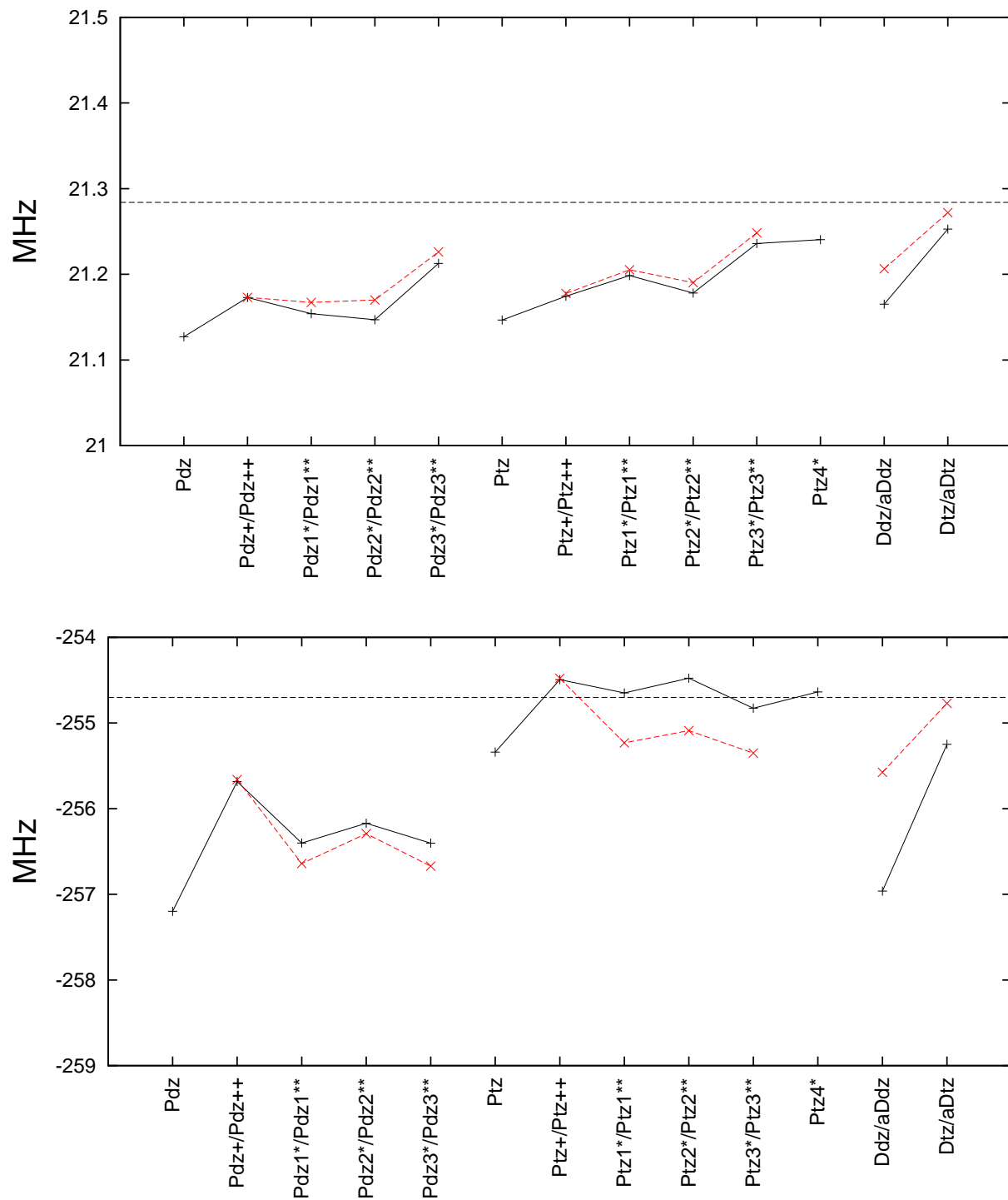


Figure 5: Calculated A_{22}^{SD} for Fe (top) and Co (bottom) versus the basis set on the non-coupled atoms. The basis set on N1–N4 (*cf.* Figure 1) is aug-cc-pVTZ-J. The (+) symbols indicate that diffuse or polarization functions are not used on hydrogen, while (x) includes additional diffuse and/or polarisation functions on hydrogens. For details of the labels see Table 1.

are added for the non-hydrogen atoms. However, due to the larger value of $A_{22}^{\text{SD}}(\text{Fe})$, this threshold corresponds to a deviation of only 0.5%. If one is satisfied with a deviation of 1% or even a maximum deviation of 0.5 MHz the choice of basis set on the non-coupled atoms has no effect.

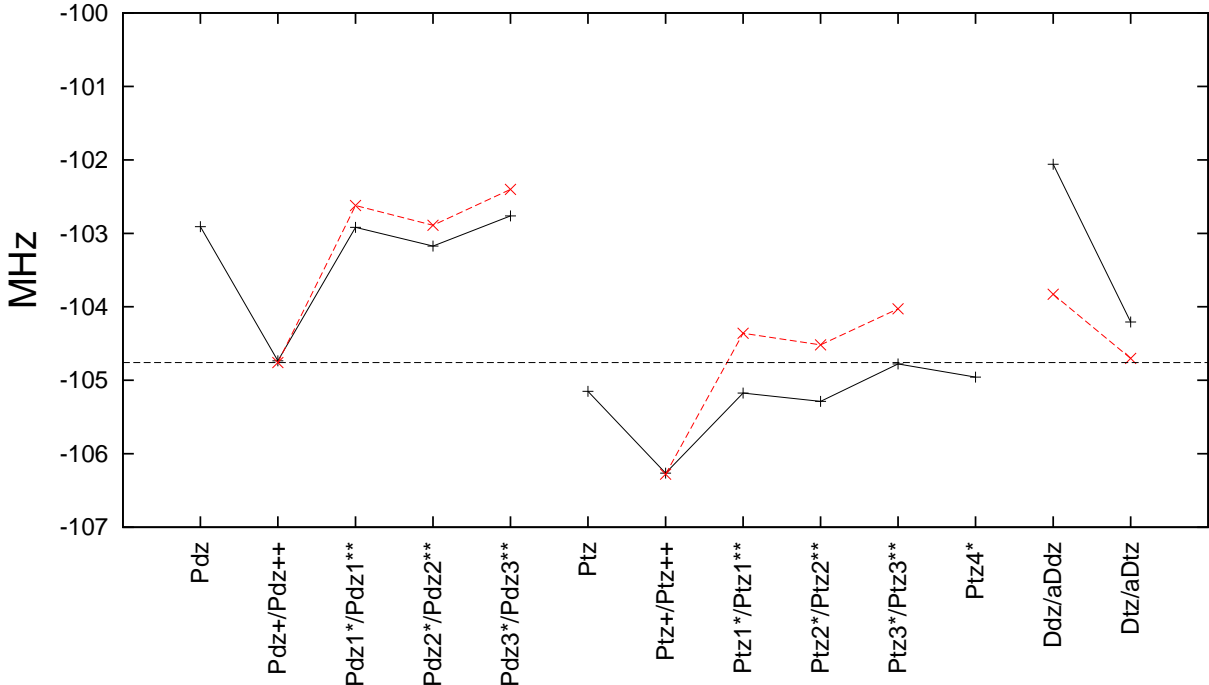


Figure 6: Calculated $A_{11}^{\text{SD}}(\text{Co})$ versus the basis set on the non-coupled atoms. The basis set on N1–N4 (*cf.* Figure 1) is aug-cc-pVTZ-J. The (+) symbols indicate that diffuse or polarization functions are not used on hydrogen, while (x) includes additional diffuse and/or polarisation functions on hydrogens. For details of the labels see Table 1.

Turning to the Co hyperfine couplings in model system **IIa** we note, that with the reference basis set the A_{11}^{SD} -component of the spin-dipolar tensor of Co is -104.76 MHz and thus almost twice as large as the $A^{\text{FC}}(\text{Co})$ term and about seven times as large as for the $A_{11}^{\text{SD}}(\text{Fe})$. The variation with the basis set is also more pronounced (see Figure 6 and Table S6 in the Supporting Material) and the variation spans in total over 6 MHz. Moreover, there seems to be no convergence towards the reference value for the valence double-zeta Pople style basis sets apart from a coincidental agreement of the 6-31+G and 6-31++G basis sets. Instead

the results seem to converge towards a less negative value which differs by 2 MHz or 2% from the reference value. The Pople valence triple-zeta basis sets, on the other hand, *do* converge towards the reference value. In fact all of the triple-zeta results, with the exception of the 6-311+G and 6-311++G basis sets, are within 1% of the reference value. Also the results of the correlation consistent basis sets vary a lot between valence double- and triple-zeta, but the valence triple-zeta results reach the reference value within 0.6 MHz or 0.6%, which can be greatly improved to 0.05 MHz by adding augmented functions.

For the 22-component of the spin-dipolar tensor, which for the reference basis set equals -254.70 MHz, the same pattern as for the 11-component is repeated, as can be seen in Figure 5 or Table S7 in the Supporting Material. In absolute values the deviations are quite similar for a given basis set, but percentwise they are only half as large as for the A_{11}^{SD} due to the larger value of the 22-component. Again there is a large difference between using a valence double-zeta or triple-zeta basis for both Pople style and correlation consistent basis sets. However, contrary to the 11-component the valence double-zeta results are now all too negative compared to the reference value by more than 1.5 MHz. All of the valence triple-zeta basis sets give results within 0.6 MHz or 0.25% deviation, but we observe that adding first and second polarisation functions does not lead to a monotonic convergence towards the reference values. Finally, the 33-component, $A_{33}^{\text{SD}}(\text{Co}) = - (A_{11}^{\text{SD}}(\text{Co}) + A_{22}^{\text{SD}}(\text{Co}))$, is the largest diagonal element, but has the smallest basis set dependence as the deviations of the 11- and 22-components mostly cancel each other.

Using the 6-31G-Juc basis set on N1–N4: The basis set dependence of the $A_{11}^{\text{SD}}(\text{Fe})$ and $A_{22}^{\text{SD}}(\text{Fe})$ terms are more pronounced when using the 6-31G-Juc basis set on the nitrogen atoms than with the aug-cc-pVTZ-J as can be seen in Figure 7 (and in Tables S8 and S9 in the Supporting Material). However, as soon as polarisation functions are added to the basis sets the deviations from the reference basis set are comparable to the deviations found with the aug-cc-pVTZ-J basis sets on the nitrogen atoms and so this smaller basis set can also be

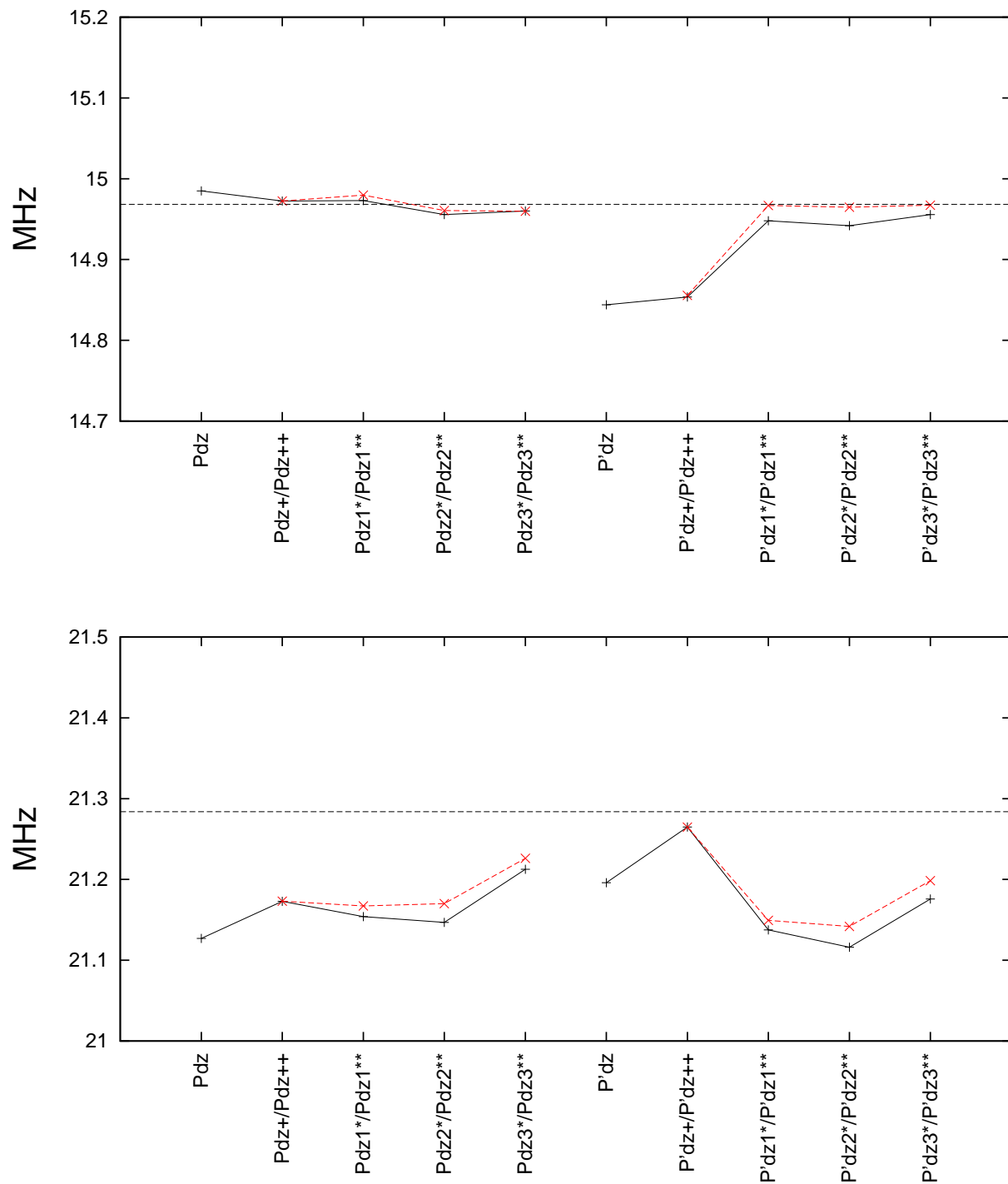


Figure 7: Calculated $A_{11}^{SD}(\text{Fe})$ (top right) and $A_{22}^{SD}(\text{Fe})$ (bottom right) with various diffuse and polarisation functions added to the 6-31G-Juc basis set on N1–N4 (*cf.* Figure 1) and to the 6-31G basis set on the non-coupled atoms. For details of the labels see Table 1. The results for aug-cc-pVTZ-J on the N atoms are shown on the left for comparison. The (+) symbols indicate that diffuse or polarization functions are not used on hydrogen, while (x) includes additional diffuse and/or polarisation functions on hydrogens.

used instead of the more expensive aug-cc-pVTZ-J for calculations of the spin-dipolar terms.

3.1.3 Second-order paramagnetic spin-orbit contribution

Using the aug-cc-pVTZ-J basis set on N1–N4: In model system **Ia** the isotropic second-order paramagnetic spin-orbit contribution $A^{\text{PSO}}(\text{Fe})$ is about a factor of 4 smaller than the Fermi contact contribution and has the opposite sign. With the reference basis set one obtains at the B3LYP level 3.76 MHz. The paramagnetic spin-orbit contribution is a second order property and its basis set dependence differs thus from the other two contributions as can be seen from Figure 8 or of the values in Table S10 in the Supporting Material. First of all, the values do not converge as monotonically and secondly the deviation from the reference result falls only below the 0.1 MHz threshold for valence triple set basis sets or by adding diffuse functions like in the 6-31+G or aug-cc-pVDZ basis sets. However, if one requires a maximum deviation of 1% or less then one would have to employ truly large basis sets like 6-311G(2df,2pd), cc-pVTZ or aug-cc-pVTZ due to the smaller absolute value of the second order paramagnetic spin-orbit contribution. Furthermore, all basis sets give results within 0.5 MHz of the reference.

With 71.49 MHz for the reference basis set the second-order paramagnetic spin-orbit contribution $A^{\text{PSO}}(\text{Co})$ is larger in model system **IIa** than the corresponding Fermi contact contribution $A^{\text{FC}}(\text{Co})$. This is thus very different from the paramagnetic spin-orbit contribution to the hyperfine coupling of Fe, but similar to Fe it has also the opposite sign of the $A^{\text{FC}}(\text{Co})$ contribution. The variation with the basis set on the non-coupled atoms, however, is much smaller than for the Fermi contact term as seen in Figure 8 and Table S11. The maximum deviation from the reference value is 0.2 MHz or 0.3%, which is thus comparable to the maximum deviation in model system **Ia** in absolute values but certainly not in percentage. Also there is a more significant effect of adding polarisation functions also on the hydrogen atoms, which in general decreases the deviation to within 0.1 MHz.

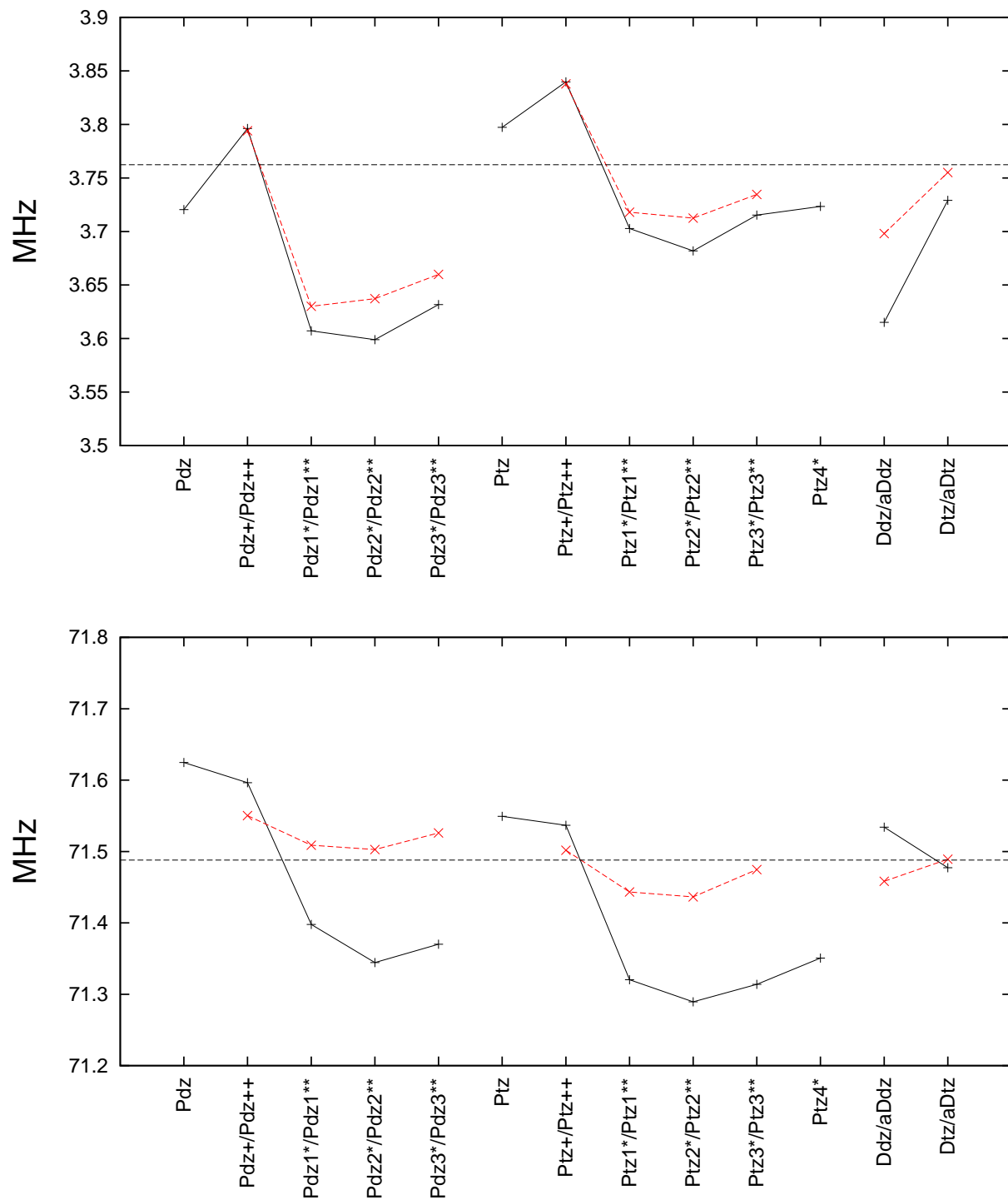


Figure 8: Calculated A^{PSO} for Fe (top) and Co (bottom) versus the basis set on the non-coupled atoms. The basis sets on N1–N4 (*cf.* Figure 1) is aug-cc-pVTZ-J. The (+) symbols indicate that diffuse or polarization functions are not used on hydrogen, while (x) includes additional diffuse and/or polarisation functions on hydrogens. For details of the labels see Table 1.

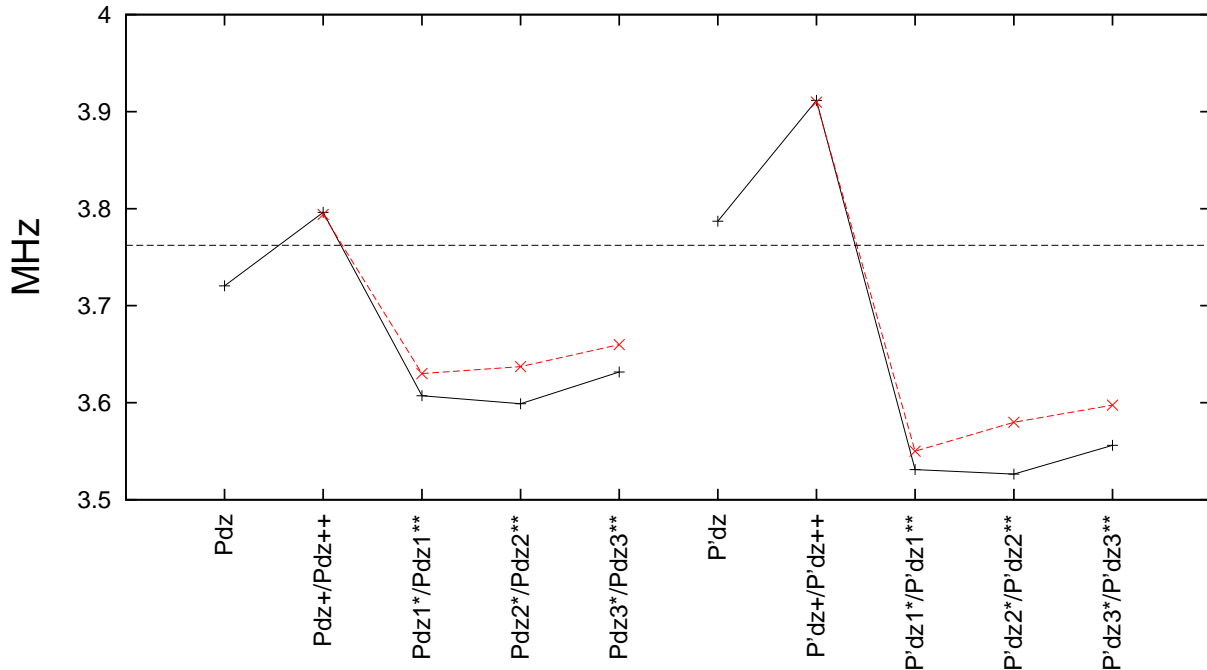


Figure 9: Calculated $A^{\text{PSO}}(\text{Fe})$ (right) with various diffuse and polarisation functions added to the 6-31G-Juc basis set on N1–N4 (*cf.* Figure 1) and to the 6-31G basis set on the non-coupled atoms. For details of the labels see Table 1. The results for aug-cc-pVTZ-J on the N atoms are shown on the left for comparison. The (+) symbols indicate that diffuse or polarization functions are not used on hydrogen, while (x) includes additional diffuse and/or polarisation functions on hydrogens.

Using the 6-31G-Juc basis set on N1–N4: The basis set dependence of the $A^{\text{PSO}}(\text{Fe})$ term with the 6-31G-Juc (augmented with the same polarization functions as on the non-coupled atoms) core property basis set on the coupled nitrogen atoms is very similar to the one found when using instead the aug-cc-pVTZ-J basis set on the nitrogen atoms as soon as polarisation functions are added to the Pople style basis sets, as can be seen from the results in Table S12 in the Supporting Material. Consequently, the deviation from the reference basis set is within 0.2 MHz which amounts to about 5% due to the small absolute value of $A^{\text{PSO}}(\text{Fe})$ (for the reference, $A^{\text{PSO}}(\text{Fe})$ is 3.76 MHz). As seen in Figure 8 a valence triple-zeta basis set is required on N1–N4 and the non-coupled atoms for a better agreement with the reference and we have only employed polarised double-zeta basis sets in our investigation with the 6-31G-Juc core property basis sets.

3.2 Superhyperfine coupling of N1–N4

In model system **Ia** the four nitrogens (N1–N4 in Figure 1) are found to give slightly different coupling constant contributions as exemplified here by the A^{FC} values of -4.86 MHz (N1), -5.17 MHz (N2), -4.99 MHz (N3), -5.41 MHz (N4) for the reference basis set. But even though we find four different super-hyperfine couplings, there appears to be only two different basis set dependencies for the N1/N2 and N3/N4 pairs. Also in model system **IIa**, the Co-complex $[\text{Co}(\text{NH}_3)(\text{CN})(\text{en})_2]^{1+}$, the four nitrogens have different super-hyperfine couplings and are paired. However, in contrast to the Fe-complex, the differences between the pairs are much larger as two of the nitrogens have positive couplings of about 26 MHz, while the other two have small and negative couplings of about -1.5 MHz. Furthermore, it is now the nitrogens, which are diagonally across from each other, N1/N3 and N2/N4, that form pairs in the Co-complex and therefore exhibit a similar basis set dependence. Since the A^{SD} and A^{PSO} terms for N1–N4 in model system **Ia** are negligible in size compared to the A^{FC} contribution we will not describe these contributions in details. Instead the reader is referred to Tables S4, S5 and S10 in the Supporting Material. Also for model system **IIa** the A^{PSO} contribution is negligible and the A^{SD} contributions are either negligible or they do not deviate with the choice of basis sets (see Tables S6, S7 and S11). For the Fermi contact contribution, Table S1 in the Supporting Material shows all the calculated Fermi contact values for the nitrogens in the Fe-complex, model system **Ia**. Both with aug-cc-pVTZ-J and 6-31G-Juc basis sets on N1–N4, the variations for $A^{\text{FC}}(\text{N})$ with the basis set on the non-coupled atoms are generally small. Also the absolute value is small, and so to obtain a deviation of less than 1% from the reference value, only valence triple-zeta basis sets or double-zeta basis sets with two polarisation functions will be sufficient. For the 6-31G-Juc basis set, the deviation from the result with the reference basis set is found to be less than 0.1 MHz for all basis sets (less than 2%). Interestingly, the results obtained when using either the aug-cc-pVTZ-J or 6-31G-Juc for N1–N4 varies quite little from each other (less than 0.1 MHz), meaning that the core property Pople based 6-31G-Juc basis sets are a good, smaller alternative for the

aug-cc-pVTZ-J basis set within the first coordination sphere.

4 Calculations on two larger model systems

In order to illustrate the performance of our basis set combinations we have calculated also the hyperfine coupling of the metal atoms and the super-hyperfine couplings of the ligand nitrogen atoms in the two larger modelsystems, **Ib** and **Ib**, of the active site of the compound 0 intermediate of cytochrome P450_{cam} and for the EPR active intermediate of coenzyme B₁₂ cyanocobalamin complex. The calculations were performed with the aug-cc-pVTZ-Juc basis set for iron and cobalt, the 6-31G(d) Pople basis set for the non-coupled atoms and either the aug-cc-pVTZ-J or 6-31G(d)-Juc basis set for the porphyrin or corrin nitrogens. The basis sets will be called Pd_z1* and P'd_z1* in the following in accordance with Table 1. All calculations were performed with the B3LYP functional and the results are given in Table 2.

It can clearly be seen in Table 2 that there is little difference between choosing the larger aug-cc-pVTZ-J or smaller 6-31G(d)-Juc basis set for the porphyrin or corrin nitrogens. Only the $A^{\text{FC}}(\text{Co})$ contribution is affected with more than 0.1 MHz (more precisely there is a 3 MHz difference). This emphasises once more that the smaller Pople based 6-31G-Juc basis sets can indeed be used for the porphyrin or corrin nitrogens instead of the larger aug-cc-pVTZ-J without sacrificing accuracy, also for much larger systems.

Many investigations have been carried out on the different intermediates in the catalytic cycle of cytochrome P450_{cam}, which includes both EPR and ENDOR studies of the hydroperoxy species done by Davydov *et al.*^{10,12} They isolated the individual substrate free P450_{cam} intermediates by means of cryoreduction at 77 K. In their early investigation they found a super-hyperfine coupling constant at the \mathbf{g}_1 -component of the \mathbf{g} -tensor for the porphyrin ¹⁴N atoms of $A(\text{N}) = 5.8 - 5.9$ MHz,¹⁰ but later experiments showed that there are two types of nitrogen with couplings of $A(\text{N}) = 5.38$ MHz and $A(\text{N}) = 7.08$ MHz at the \mathbf{g}_1 .¹² We can compare this with our A^{FC} contributions in Table 2 as the SD and PSO are too small to be

Table 2: Contributions to the hyperfine coupling constant of the central metal atom and to the super-hyperfine coupling constants (in MHz) of the porphyrin or corrin nitrogens in model systems **Ib** and **IIb**. Note that $A_{33}^{\text{SD}} = -A_{11}^{\text{SD}} - A_{22}^{\text{SD}}$. For information on the basis set abbreviations see Table 1.

Atom	A^{FC}		A_{11}^{SD}		A_{22}^{SD}		A^{PSO}	
	Pdz1*	P'dz1*	Pdz1*	P'dz1*	Pdz1*	P'dz1*	Pdz1*	P'dz1*
Ib								
Fe	-16.47	-16.54	13.70	13.74	22.30	22.31	3.53	3.45
N1	-5.94	-5.91	0.14	0.15	-0.04	-0.04	0.03	0.03
N2	-6.23	-6.19	0.04	0.05	0.06	0.06	0.04	0.04
N3	-6.10	-6.05	0.18	0.19	-0.03	-0.03	0.03	0.03
N4	-5.74	-5.68	0.16	0.17	0.01	0.01	0.03	0.03
IIb								
Co	77.63	74.60	-208.36	-208.79	-152.23	-152.26	81.84	81.60
N1	-4.07	-4.05	0.98	0.98	-0.19	-0.18	0.08	0.08
N2	-2.54	-2.50	1.00	1.02	-0.17	-0.17	0.05	0.05
N3	-3.66	-3.62	1.08	1.09	-0.38	-0.39	0.07	0.07
N4	-4.44	-4.39	0.66	0.67	-0.02	-0.01	0.07	0.07

relevant. Our calculated values rang from -5.7 MHz and -6.2 MHz for the four nitrogens in the model system **Ib**. For the hyperfine coupling of the Co atom of coenzyme B₁₂ in e.g. ethanolamine deaminase⁵⁸⁻⁶⁴ an isotropic value of 123 MHz has been reported,⁶² which can be compared with our result ≈ 160 MHz for model system **IIb**. Both our calculated values are in reasonable agreement with the experimental values, if one keeps in mind that we have not investigated the effect of optimising the choice of DFT exchange-correlation functional nor of the protein environment. Both can be expected to have large effects but a systematic investigation of this is beyond the scope of this study.

5 Summary and conclusions

The possibility to employ locally dense basis sets, *i.e.* proper core property basis sets on the coupled atoms and smaller basis sets on the non-coupled atoms, in the calculations of EPR hyperfine coupling constants, was investigated at the DFT level for a model system of the Fe containing active site of the compound 0 intermediate of Cytochrome P450_{cam}, [Fe(OOH)(SH)(en)₂]⁺ or model **Ia**, and for a model of the Co containing coenzyme B12, [Co(NH₃)(CN)(en)₂]⁺ or model **IIa**. As core property basis sets for the metal atoms, Fe and Co, the newly developed aug-cc-pVTZ-J basis sets were used in their uncontracted form, while for the N atoms of the ethylenediamine equatorial ligands the aug-cc-pVTZ-J or an uncontracted version of the newly developed 6-31G-J basis sets were used. For the non-coupled atoms either smaller correlation consistent or Pople style basis sets were employed.

In conclusion to the basis set study we find that using either any of the smaller correlation consistent basis sets or Pople style basis sets with one or sometimes two polarisation functions on the non-coupled atoms, give acceptable results. This leads to almost a bisection of the size of the basis sets for the two model systems without significantly reducing the accuracy of the results. For the largest contribution, the Fermi contact term, one obtains a deviation of about 1% or less from the reference basis set with all correlation consistent basis sets or with Pople basis sets as small as the 6-31G(d) (6-31G(2d) for Co). For Fe in model system **Ia** the deviation is even less than 0.1 MHz from the reference value with any polarised basis set on the non-coupled atoms and all basis sets give reasonable Fermi contact contribution results within 0.5 MHz accuracy. Only the A_{22}^{SD} and A^{PSO} contributions deviate for iron with more than 0.1 MHz from the reference value and even this can be reduced to less than 0.1 MHz by using the valence triple-zeta 6-311G(d) basis sets for the non-coupled atoms. For spin-dipolar contributions to the hyperfine coupling tensor of Co in model system **IIa** a 1% accuracy requires again polarised valence triple-zeta basis sets like 6-311G(d), while for the paramagnetic spin-orbit contribution this is obtained with all basis sets included in this study.

For the nitrogen hyperfine couplings, calculated with either the aug-cc-pVTZ-J basis set or with an uncontracted version of the basis sets based on the 6-31G-J basis set, most basis sets on the non-coupled atoms give results within 0.1 MHz accuracy of the reference basis for the Fermi contact term and all basis sets for the other contributions. Therefore, it will be sufficient to describe the core electrons of the nitrogens with the smaller core property 6-31G-J basis set both when calculating the hyperfine coupling of iron as well when calculating the super-hyperfine coupling of the porphyrin nitrogens in future studies of the active site in cytochrome P450_{cam}.

Calculations with our locally dense basis sets of the hyperfine and super-hyperfine coupling constants of a typical gas phase model system for the active site in the compound 0 intermediate of cytochrome P450_{cam} show good agreement with the available experimental data.

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