An Integrated Computational Approach to the Elctron Paramagnetic Resonance Characterization of Rigid 3_{10}-Helical Peptides with TOAC Nitroxide Spin Labels

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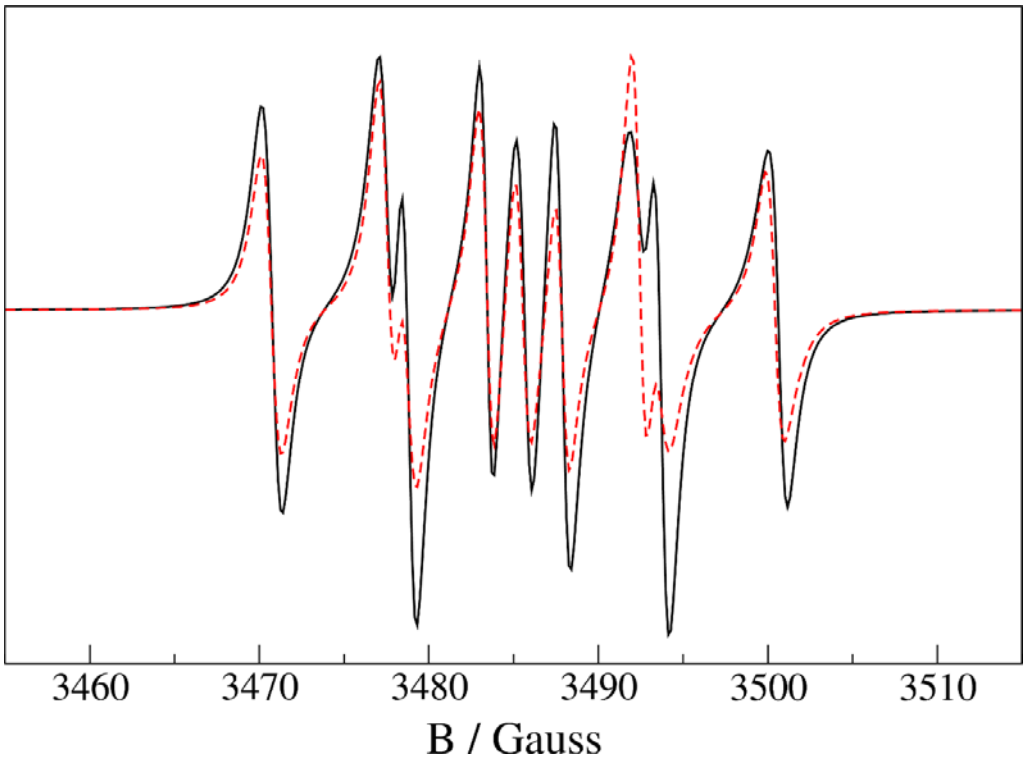
SUPPORTING INFORMATION

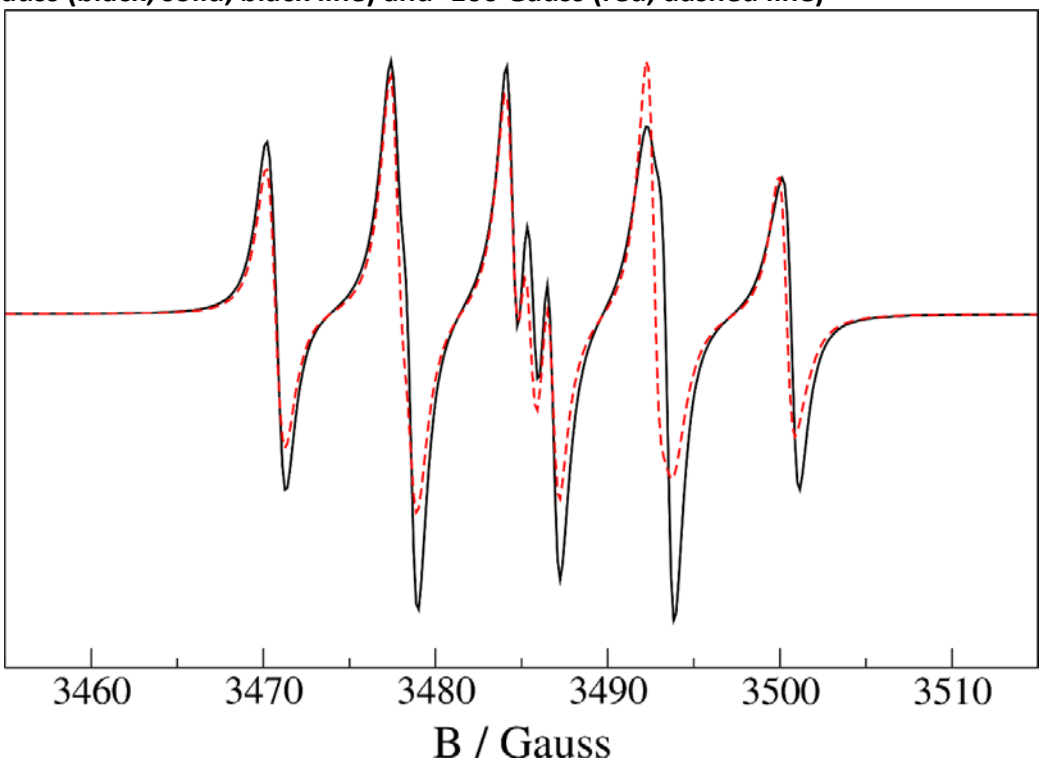
S1. Effect of the sign of *J*

The following figures show the comparison of purely theoretical spectra calculated with the same parameters as those of the $NONA_{2,8}$ *bis*-radical peptide, but imposing three different "artificial" values of $|J|$ = 50, 100, and 200 Gauss. We compare the spectra obtained with the positive values of the coupling constant (black, solid line) and the spectra obtained with the negative values (red, dashed line) of the coupling constant. These theoretical plots show how the sign of *J* influences the inhomogeneous line broadening of the peaks in all the range of sensitivity of the spectra on this parameter.

J = +50 Gauss (black, solid line) and -50 Gauss (red, dashed line)



J = +100 Gauss (black, solid, black line) and -100 Gauss (red, dashed line)



J = +200 Gauss (black, solid line) and -200 Gauss (red, dashed line)

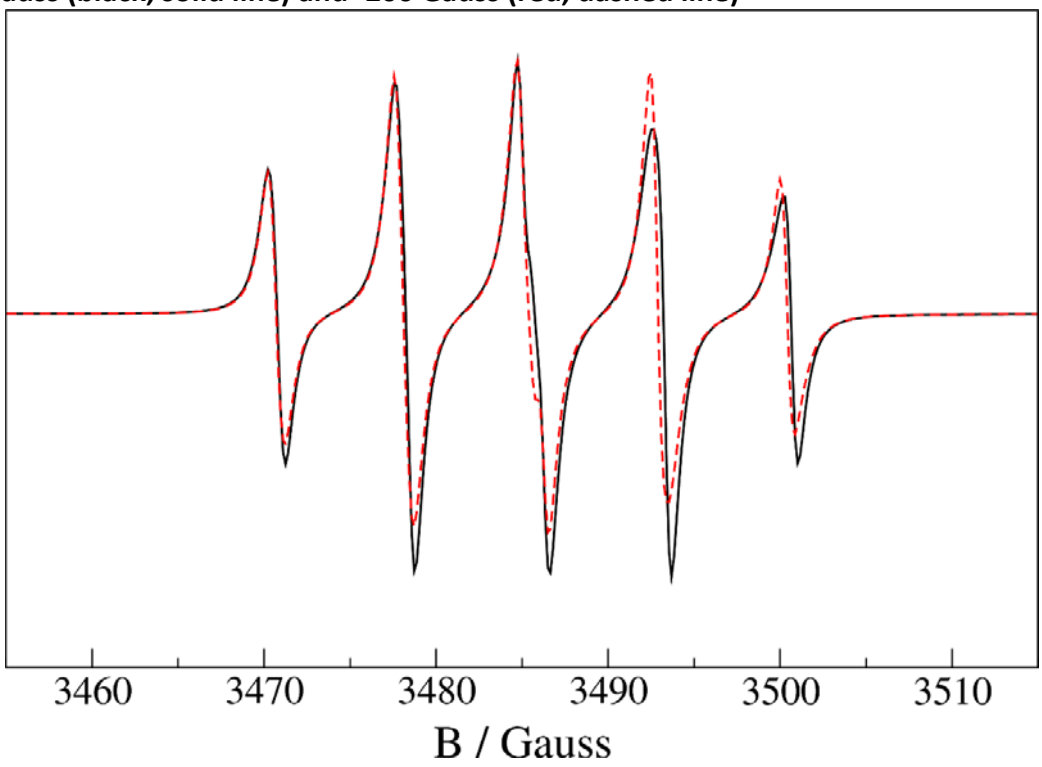


Figure S1. Simulations showing the effect of the change in sign of *J* for different values of |*J*|. Red, dashed lines: negative sign of *J*, black, solid lines: positive sign of *J*.

S2. Significance of small *J* and of its sign

From the experimental spectra of $OCTA_{2,7}$ and $NONA_{2,8}$ *bis*-radicals it is evident that the *J* coupling between the two unpaired electrons is small in absolute value. In our present approach, *J* is not calculated *a priori*, rather it is considered as a fitting parameter, together with the intrinsic linewidth, γ. Since both the parameters provide a similar, small, contribution to the broadening of the lines, it is necessary to establish if the "existance" of *J* is significant and, secondly, if the sign does also makes sense.

To answer the first question, we performed an F-test comparison between two models to fit the experimental data. In the first model, that we shall call model 1, only the intrinsic linewidth has been fitted, keeping *J* = 0. In the second model (model 2), instead, we include also *J* in the fitting. The table below reports the values of the χ^2 for both the models (which have been applied to fit spectra of both the peptides) as well as the best fitting parameters and the number of degrees of freedom (d.o.f.) in the fitting. The latter, are calculated as the number of experimental data points (2048) minus the number of fitting parameters.

Peptide	Model 1 (d.o.f. = 2047)		Model 2 (d.o.f. = 2046)		
	γ / Gauss	χ^2	γ / Gauss	*J* / Gauss	χ^2
$OCTA_{2,7}$	0.609	3.1594	0.418	-0.380	1.7811
$NONA_{2,8}$	0.523	3.5173	0.443	+0.308	2.2071

Using data in the previous table, the F values of the $OCTA_{2,7}$ and the $NONA_{2,8}$ peptides are, respectively 2386 and 1215. Using F-test tables for F(1, 2046) distribution, one obtains that there is a probability $P < 0.001$ that if model 1 is correct, model 2 fits data better than model 1 only by chance. Thus, we can conclude with high confidence that model 2 is the correct one. This, in turn, means that a value of *J* different from zero, even if very small, has physical significance.

To establish if the sign makes sense, we evaluated the error on both *J* and γ from the fitting using the diagonal parts of the covariance matrices (which are given in output from E-SpiReS). For the two fits, these matrices read

$$\mathbf{C}_{\mathrm{OCTA}_{2,7}} = \begin{bmatrix} 1.109392\cdot10^{-5} & 8.501197\cdot10^{-6} \\ 8.501197\cdot10^{-6} & 1.920487\cdot10^{-5} \end{bmatrix},$$

and

$$\mathbf{C}_{\mathrm{NONA}_{2,8}} = \begin{bmatrix} 2.667103\cdot10^{-5} & -1.297898\cdot10^{-5} \\ -1.297898\cdot10^{-5} & 2.091513\cdot10^{-5} \end{bmatrix}.$$

for the parameters expressed in Gauss. In the matrices, $C_{1,1}=\sigma_J^2$, $C_{2,2}=\sigma_\gamma^2$, and $C_{1,2}=C_{2,1}=\sigma_{J,\gamma}^2$. The estimated error on both *J* and γ is 1%. Thus, we can conclude that the sign is determined by the fitting due to the very small error on *J*, which in turn means that there is a relevant change in the χ^2 function due to small changes of *J*.

Finally, using the non-diagonal elements of the covariance matrices we calculated the correlation

coefficient between the two fitting parameters. They are 0.58 and -0.55 for the $OCTA_{2,7}$ and $NONA_{2,8}$ peptides, respectively. This means that there is a moderate (expected) correlation between *J* and γ. This correlation, based on the sign, means that an increase, in absolute value, of J corresponds to a decrease of γ. However, there is a net physical difference between the two parameters: *J* coupling affects each line in the spectrum differently, whereas γ broadens each line by the same amount. Fitting with model 1 tells that a broadening of 0.5 / 0.6 Gauss is required. With model 2, part of this broadening is caused by *J*. Just for completeness, a fit of $NONA_{2,8}$ spectrum with $\gamma = 0$ (i.e., only *J* fitted, with best fit value 0.38 Gauss) leads to bad agreement as seen in the following figure (red, solid line is the experiment, while black, dashed line the best fit). This means that *J* is not sufficient to account for all the residual line broadening and some effects are neglected (e.g., the hyperfine coupling with the protons of the TOAC-methyl groups).

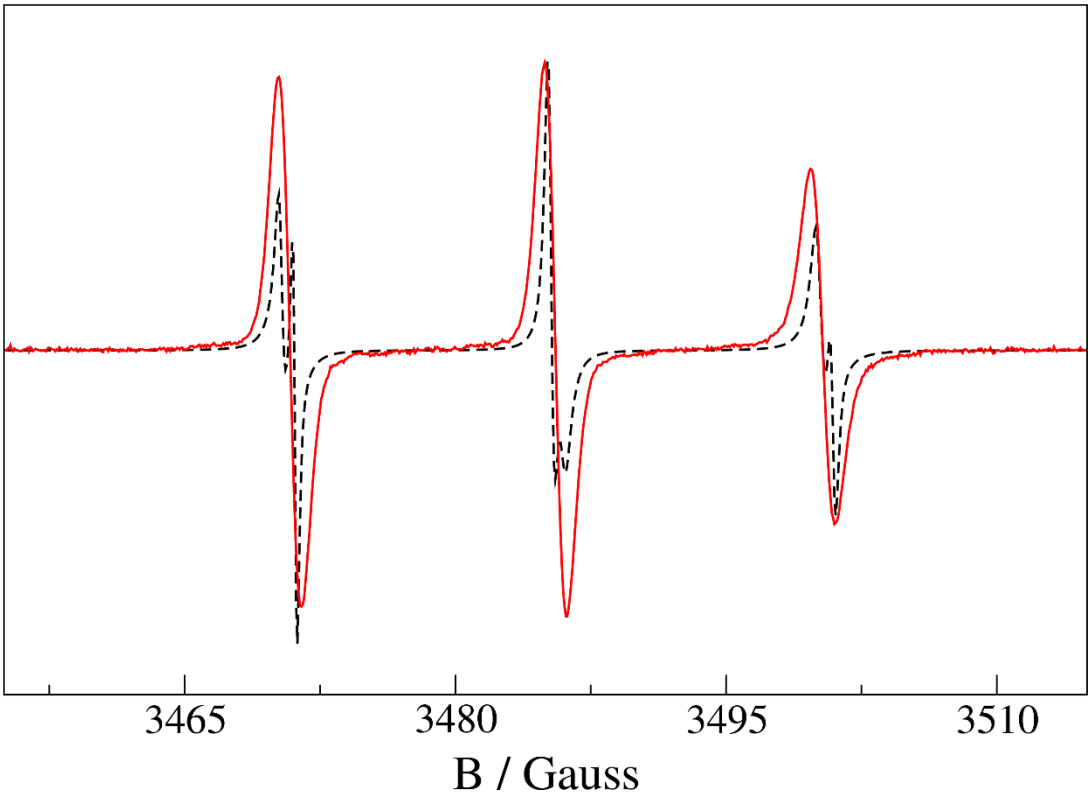


Figure S2. Simulations showing the fitted spectra with γ = 0 and only *J* fitted. Red, solid line: experimental spectrum; black, dashed line: best fit theoretical spectrum.

In conclusion, considering the good outcome of F-test, the small error in the fitting parameters, and their different physical meaning, we believe that the ICA applied to the interpretation of experimental spectra of *bis*-radicals is a reliable method to obtain *J* values and their sign from experimental spectra, even for very small $|J|$.

S3. Estimation of errors of parameters

Errors on the parameters reported in Tables 2 and 3 in the main text have been roughly evaluated as follows.

1) Errors of the intrinsic linewidth, γ, and J have been determined from fitting to be of the order of 1% as discussed in the previous Section of this SI document.
2) Error of the tensors ***g*** and ***A*** are evaluated from the "resolution" in field of the spectra, i.e. the distance in field between two points in the experimental spectrum, which is 0.07 Gauss. We chose such a way since the isotropic parts of both the tensors have been extracted from the spectrum. Thus, for ***A*** we take 0.07 Gauss as the absolute error and thus a relative error of ±0.5% is employed. For ***g***, the relative error on the isotropic part of the tensor with respect to the free electron g_e, derived from the 0.07 Gauss resolution of the spectrum, is about $5.0 \cdot 10^{-6}$. Thus the relative error is ±0.1%. These errors should be propagated on the orientations of the tensors starting from one matrix representation of the tensor and solving the eigensystem. Such a procedure is quite complicated. Rather, we chose to associate an absolute error to the Euler angles based on the sensitivity of the spectrum to their values. This sensitivity can be settled to 0.1 deg.
3) Error on the distance of the nitroxides: the distance has been evaluated from PDB files, where coordinates are usually reported in Å with three decimal figures. We estimate an error of the order of 0.1 Å for the distance.
4) Error on the diffusion tensor: assuming that the viscosity is known at high precision, the error on the values of the diffusion tensor is proportional to that on the temperature. Assuming a 1 K error on the temperature control during the experiment, and a diffusion tensor of the order of 10^8 Hz, we estimate an error of 0.3% on the components. For what concerns the Euler angles, again we select 0.1 deg, as discussed above.