

Tyrosyl Radical Spectra Simulation Algorithm: Frequently Asked Questions

TRSSA,¹ as any new method, often evokes questions. This document addresses some typical questions which we are asked most frequently

Q.: How could we use TRSSA in finding the tyrosine on which the observed radical is located in a protein?

A.: **Firstly**, the EPR spectrum of the radical (X-band or HF or even better - both) should be simulated by using any EPR spectra simulation software but the parameters of the simulation should be found by TRSSA.¹ The algorithm uses only two input variable, ρ_{C1} and θ , and calculates 12 simulation parameters according to the semi-empirical dependences (another 18 parameters are set invariant by TRSSA for all tyrosyl radicals). Simulations employing TRSSA are therefore set on a much smaller space of variables. If a spectrum cannot be simulated by TRSSA, it is highly unlikely that the radical is on a tyrosine. If a spectrum, on the other hand, is accurately simulated, the ρ_{C1} , and θ values used as the TRSSA input are the accurately determined tyrosyl radical parameters. **Secondly**, angle θ should be determined for all tyrosine residues in the protein's structure. This task can be performed by using our on-line database (<http://privatewww.essex.ac.uk/~svist/lev1/tyrdb/home.shtml>). The database analysis yields a shortlist of tyrosine residues with angles θ most close to the θ -value determined from the simulation. Those residues are the most likely candidates to host the radical. **Thirdly**, the other TRSSA input parameter, ρ_{C1} , is also an asset. If its value is high, this indicates that the radical is hydrogen bonded (the higher ρ_{C1} , the stronger the H-bond). Sometimes this allows excluding from consideration those tyrosines from the shortlist which do not form hydrogen bonds. Also, by performing the DFT calculations for the tyrosine-candidates in their individual environment, the isotropic components of the hyperfine splitting constants of the methylene protons can be found; from those – the McConnell spin density ρ_{C1} can be determined for different tyrosines and compared with the value used in the simulation. Thus, spectra simulation using TRSSA provides two ways of identification the tyrosine residue: by angle θ and by McConnell spin density ρ_{C1} .

Q.: Why is TRSSA better than previous methods of radical spectra simulation?

A.: TRSSA is much more accurate. It makes use of previously reported simulation parameters which are processed in an elaborate way and effectively averaged on the space of all known X-band and HF EPR spectra of tyrosyl radicals. As a result the accuracy is increased. According to the literature, ρ_{C1} varies within a range of 0.14–0.49. TRSSA establishes for the first time a five-fold smaller range for ρ_{C1} in the tyrosyl radicals observed so far, 0.350–0.420.¹ No other method of spectra simulation can be accurately applied to all tyrosyl radicals. In fact, each spectrum simulation published by other authors is specific for the tyrosyl radical particularly studied, the approach breaks down if somebody is going to use it (as a method) to simulate other tyrosyl radical spectra. TRSSA is the only globally consistent way to simulate the spectra, and it is here that its high accuracy is coming from.

Q.: How important is this high accuracy of TRSSA? Does it have implications on conclusions possibly drawn from simulation?

A.: Lack of accuracy often results in incorrect conclusions. DS has pointed before² to an error in assigning the EPR spectrum evolution in PGHS to ring rotation.³ He has now strengthened his arguments by more rigorous EPR spectra simulation, involving distribution of the simulation parameters in a radical population.⁴

Q.: The 2–4° accuracy in θ in a tyrosyl radical (achievable by TRSSA) seems irrelevant to the likely to be far less accurate θ values in the tyrosine residues as determined from the crystal structure. Why use TRSSA?

A.: There are tyrosine residues for which crystallography reports rather variable rotation angle (within an interval of ~35°) when compared across a number of structure files for the same protein. The EPR spectra of the radicals located on such tyrosines (e.g. in Hb¹) largely reflect the most favourable conformation, typical for a free tyrosine⁵ (phenoxy ring rotates). There are also tyrosines for which angle θ is persistently reported within a narrow range of 3°–7° (ring rotation is restricted or arrested). This accuracy fits well to the 2–4° accuracy of TRSSA and is sufficient for picking up a shortlist of tyrosines.

Q.: Radical site identification by using TRSSA requires that θ does not change much when a tyrosine is oxidised to a radical. Is such view supported?

A.: For a range of cases, though not for all, TRSSA gives the values of θ less than 3.2° different from the values measurable from the 3D structure,¹ thus showing no significant alteration of θ on tyrosine oxidation in those cases. This observation, however, cannot serve as a proof that this is always the case, and each particular case should be carefully looked into, for example by the computational chemistry methods, by running geometry optimisation of closed shell tyrosine and open shell tyrosyl radical.

Q.: TRSSA was formulated in 2004 on the basis of 11 different tyrosyl radicals for which X-band and high field EPR spectra had been reported. Has the algorithm been confirmed since that time in simulations of new tyrosyl radicals?

A.: Yes, TRSSA has been successfully used to simulate EPR spectra of the tyrosyl radical in cyt *c* complexed with cardiolipin,⁶ of the new tyrosyl radical in *B. anthracis* ribonucleotide reductase,⁷ and of the Tyr_Z radical in photosystem II.⁸

Q.: Why does TRSSA require improvement?

A.: All empirical relationships embedded in TRSSA can be calculated using the DFT methods. Some of the calculated relationships might be found very close to the empirical ones (e.g. the A_{Hβ1} and A_{Hβ2} values on θ), the others – might show only a common general trend but with notable differences in absolute values (e.g. the g-factors on ρ_{C1}). Considering the fact that there is a number of tyrosyl radicals for which both EPR and structural data are available, it will be possible to analytically link the TRSSA and DFT-calculated relationships. This would provide a general solution for the problem of simulation of an EPR spectrum (X-band and/or HF) for a radical on a specific tyrosine in a specific molecular environment. The recipe will be as follows: run the DFT calculation for the tyrosyl radical with the nearby groups within 6 Å, process the output file according to the analytical links between the TRSSA and DFT-calculated relationships, use the output for a spectrum simulation. Importance of creating such method goes beyond this proposal and into the wide area of protein based free radicals. Currently growing number of reported tryptophanyl EPR spectra would allow developing (using the same framework) a similar algorithm for tryptophanyl radicals spectra. Generalisation of the two algorithms in the future would further make possible to create an algorithm for prediction EPR spectra for any protein radical – on any residue with any immediate molecular environment.

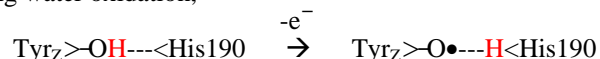
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7. Torrents E., Sahlin M., Biglino D., Graslund A. and Sjöberg B. M. (2005) Efficient growth inhibition of *Bacillus anthracis* by knocking out the ribonucleotide reductase tyrosyl radical. *Proc. Natl. Acad. Sci. U.S.A.* **102**: 17946-17951.

Our simulation of the published spectrum can be viewed at

http://privatewww.essex.ac.uk/~svist/lev1/Downloads/anthracis_RNR.pdf

8. Petrouleas V. (2007) The 40th Annual International Meeting of the Electron Spin Resonance Group of the Royal Society of Chemistry, New College, Oxford, 25-29 March 2007.

The ρ_{C1} value found from the simulation was notably high, 0.40, implying a strong hydrogen bond in which phenoxyl oxygen of the radical is involved. This corresponds well to the view promoted by Petrouleas that the special assembly of Tyr_Z, its base partner His190 and tetracuclear Mn cluster provides the core mechanism for water oxidation. The hydrogen bond between Tyr_Z and His190 is a necessary condition for effective proton translocation reactions accompanying water oxidation,



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