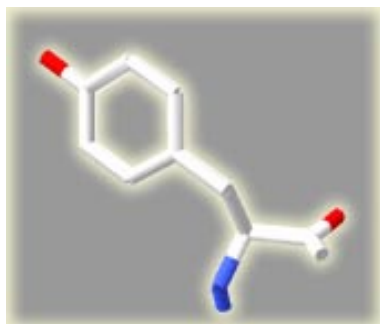




# Tyrosine residues in different proteins: Phenol ring rotation angle database

Last modified: 08.03.2004

This document on the web: <http://privatewww.essex.ac.uk/~svist/lev1/tyrdb/home.shtml>

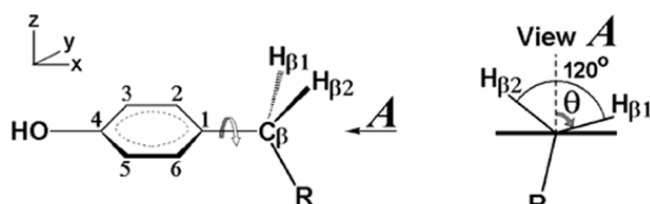
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## 1. Tyrosine is a common site of free radical formation in proteins

Tyrosine is one of the twenty amino acids used as building blocks in proteins. It is a well established fact that free radicals can be formed on proteins, and tyrosine is certainly the most frequently reported site of such free radical formation. Many enzymes make use of the *tyrosyl* radicals (the products of one electron oxidation of tyrosine) in their catalytic mechanism. On the other hand, tyrosyl radicals can be harmful when formed in an uncontrolled fashion, e.g. when a peroxide reacts with haem proteins.

When an electron is subtracted from tyrosine, a cation radical is formed which immediately (at physiological pH) drops the oxygen proton, so that the tyrosyl radical is a neutral species. The ring can rotate around the C1 - C $\beta$  bond in a free tyrosine (Fig . 1).



**Fig. 1.** This figure defines dihedral angle  $\theta$  in tyrosine. Clearly, angle  $\theta$  is just one of the ways to describe rotation of the phenol ring around the C1 - C $\beta$  bond. This definition uses view A: angle  $\theta$  is defined for proton  $\beta_1$ ; it is positive when measured clockwise from the upward perpendicular to the ring plane when the C $\beta$ -R bond is below the horizontally oriented ring plane.

Therefore, depending on specific electrostatic environment, the rotational conformation of the phenol ring will be different in different tyrosine residues in proteins.

## 2. What does an EPR spectrum tell us about the radical's conformation?

EPR spectroscopy is the method of detection of paramagnetic species. Free radicals constitute a significant class of paramagnetic species since they, by definition, have a non-zero electron spin. If a radical lives long enough - its EPR spectrum can be recorded. Very often the EPR spectrum is so specific for a particular radical that it can be considered as its signature. This is not, however, the case for the tyrosyl radicals: the EPR spectra of the tyrosyl radicals found in different protein systems are remarkably variable. The main (though not exclusive) reason for that is the ability of the phenoxyl group to rotate around the C1 - C $\beta$  bond and

the fact that the actual angle of such rotation is different in different tyrosyl radicals.

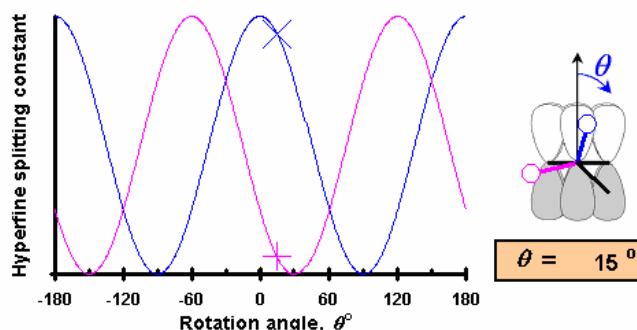
The appearance of the EPR spectrum (its lineshape) is defined by the hyperfine interaction of the unpaired electron with the radical's protons. While such interaction for the four ring protons is independent of the orientation of the ring, on which the most of the spin density resides, the hyperfine splitting constants for the  $\beta$ -protons of the methylene group do depend on the rotation angle  $\theta$  according to the McConnell relation:

$$A_{iso}^{\beta} = \rho_{C1}(B' + B''\cos^2\theta) \quad \{1\}$$

where  $A_{iso}^{\beta}$  is the isotropic hyperfine splitting constant,  $\rho_{C1}$  is the spin density on atom C1; B' and B'' are constants, B' being commonly neglected in practical applications and B'' being equal to 58 G. The  $A_{iso}^{\beta}$ -values for both  $\beta$ -protons can be determined via a simulation of the experimental X-band (~9 GHz) EPR spectrum. The two unknowns,  $\rho_{C1}$  and  $\theta$ , can then be found from a system of two equations {2}, written for the two protons.

$$\left. \begin{aligned} A_{iso}^{\beta_1} &= \rho_{C1}B''\cos^2\theta \\ A_{iso}^{\beta_2} &= \rho_{C1}(B''\cos^2(\theta - 120^\circ)) \end{aligned} \right\} \quad \{2\}$$

The graphical representation of these two equations follows in Fig. 2. You can make this figure interactive by



By [clicking on this picture](#), you could make it interactive. You should have Excel installed on your computer; its security level should be set to Medium (from the main Excel menu, go to Tools/ Macro/ Security...). You also should allow macros when prompted. Netscape and older versions of IE would not open the interactive picture here, but you still will be able to download and save on your computer the file *theta\_in\_tyr\_for\_web.xls* which could be then open by Excel. Please note that opening the interactive picture can take a little while (280 KB).

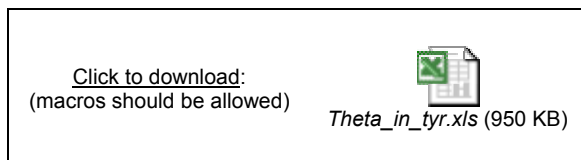
**Fig. 2.** The McConnell's dependences of the isotropic hyperfine splitting constant on the rotation angle  $\theta$  written for two methylene protons {2} are phase-shifted by 120°.

clicking on it. You will then be able to change the value of  $\theta$  and to view the conformation and the new values of the isotropic hyperfine splitting constants.

Thus, a simulation of an EPR spectrum of a tyrosyl radical provides us with the values of the isotropic hyperfine splitting constants for the methylene protons. These two values then can be used in system {2}, and the values of  $\theta$  and  $\rho_{C1}$  can be found.

## 2.1. Excel file-calculator download

The solutions of system {2} can be found numerically. I have created an Excel file (*theta\_in\_tyr.xls*) that can be used for solving system {2} on a routine basis:



This file can be used to perform two different tasks. You can find the values of  $\rho_{C1}$  and  $\theta$  for known values  $A^{\beta^1}_{iso}$  and  $A^{\beta^2}_{iso}$ . Alternatively, you can set some values for  $\rho_{C1}$  and  $\theta$  and find the values of the isotropic hyperfine constants  $A^{\beta^1}_{iso}$  and  $A^{\beta^2}_{iso}$ . I tried to make the *theta\_in\_tyr.xls* file user friendly. If, however, you have difficulties in using this file, please contact me and I will be happy to help.

## 3. About this database: linking EPR and structure

This database was created to store and to make easily accessible the information about rotational conformation of the ring in different tyrosines in different proteins. Why do we need such information?

As explained above, an EPR spectrum of a tyrosyl radicals in a protein provides very definite information about the rotational angle  $\theta$  of the phenoxy ring in the radical. Successful simulation of such EPR spectrum yields two values of the isotropic hyperfine splitting constants for the methylene protons, and angle  $\theta$  can be found from these values. This database can then be used to identify the Tyr residues in the protein which are in the conformation close to that found for the radical. Thus this database can help to assign the radical to a particular Tyr site (and there could be dozens of those in one protein!).

## 4. How to use the database

You do not have to be registered in order to search the database.

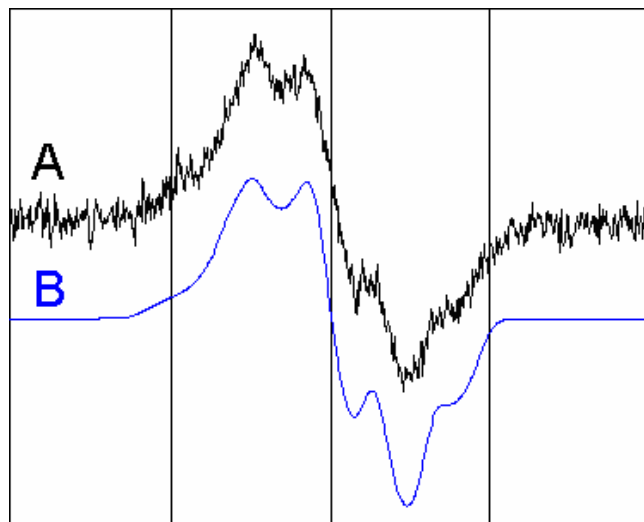
First thing to do is to specify the protein structure file. Go to 'Search' on the top of this page, and you will find two ways to do it. If you know the Protein Databank ID code of the protein (PDB ID), just select it from the pull-down menu and click 'Next'. Alternatively, you can search the records by a keyword. The screen that shows a search result for a protein will have a list of Tyr residues in a table. Ignore the  $\phi_2$  and  $\phi_6$  columns for the time being, you will only need to analysed the first three columns. The data in the table can be sorted by these columns. Since you probably want to know which Tyr residues are in a particular rotational conformation (the conformation found by EPR in the radical), you can set a value for  $\theta$  in the provided field ( $\theta_{target}$ ) and sort data in the table by the difference  $|\theta - \theta_{target}|$ . The top of the table, when sorted by this difference, shows the Tyr residues with the most close rotational conformation to that found in the radical by the EPR spectroscopy.

## 4.1. Example: Human haemoglobin (HbA) reacting with hydrogen peroxide

A tyrosyl free radical is formed in this reaction. Before actually using the database, we will have to determine the rotational conformation of the phenoxy ring in the radical.

### 4.1.1. How to determine $\theta$ in the radical

The EPR spectrum of the radical formed in the reaction of human haemoglobin with hydrogen peroxide and its simulation are shown in Fig. 3.



**Fig. 3.** **A** - an EPR spectrum of the reaction mixture of 0.71 mM (by haem) metHbA and 7.1 mM  $H_2O_2$  taken at room temperature, pH 8 (aerated condition), immediately after mixing (the middle of the spectrum corresponds to ~6 s of reaction time). The instrumental conditions were: microwave frequency, 9.798 GHz; microwave power, 1.27 mW; modulation amplitude, 2 G; time constant, 10.2 ms, sweep time, 10.5 sec; central field, 3490 G; single scan. **B** - a computer simulated spectrum performed with the software *simpow6* (Mark Nilges, Illinois EPR Research Center, <http://ierc.scs.uiuc.edu/~nilges/software.html>). The simulation parameters were as follows: microwave frequency, 9.79747 GHz; central field, 3482.25 G; field width, 80 G; modulation amplitude, 2.00 G;  $g_x = 2.00814$ ;  $g_y = 2.00433$ ;  $g_z = 2.00218$ ; individual linewidth components,  $\Delta H_x = 4.54$  G;  $\Delta H_y = 3.16$  G;  $\Delta H_z = 3.12$  G; hyperfine splitting constants (all in MHz) and Euler angles (degree):

$$\begin{aligned} A^{\beta^1}_x &= 24.34; A^{\beta^1}_y = 20.83; A^{\beta^1}_z = 20.83; \phi^{\beta^1} = 0^\circ; \\ A^{\beta^2}_x &= 12.36; A^{\beta^2}_y = 7.73; A^{\beta^2}_z = 7.73; \phi^{\beta^2} = 0^\circ; \\ A^{C^3}_x &= -25.6; A^{C^3}_y = -8.0; A^{C^3}_z = -19.1; \phi^{C^3} = 22^\circ; \\ A^{C^5}_x &= -27.5; A^{C^5}_y = -8.0; A^{C^5}_z = -20.5; \phi^{C^5} = -22^\circ; \\ A^{C^2}_x &= 5.0; A^{C^2}_y = 7.5; A^{C^2}_z = 1.5; \phi^{C^2} = 10^\circ; \\ A^{C^6}_x &= 5.0; A^{C^6}_y = 7.5; A^{C^6}_z = 1.5; \phi^{C^6} = -10^\circ. \end{aligned}$$

From the values for the hyperfine splitting constants for the methylene protons, used in the simulation,

$$A^{\beta^1}_x = 24.34 \text{ MHz}; A^{\beta^1}_y = 20.83 \text{ MHz}; A^{\beta^1}_z = 20.83 \text{ MHz};$$

$$A^{\beta^2}_x = 12.36 \text{ MHz}; A^{\beta^2}_y = 7.73 \text{ MHz}; A^{\beta^2}_z = 7.73 \text{ MHz};$$

the isotropic values can be found:

$$A^{\beta^1}_{iso} = (A^{\beta^1}_x + A^{\beta^1}_y + A^{\beta^1}_z) / 3 = (24.34 + 20.83 + 20.83) / 3 = 22.00 \text{ MHz}$$

$$A^{\beta^2}_{iso} = (A^{\beta^2}_x + A^{\beta^2}_y + A^{\beta^2}_z) / 3 = (12.36 + 7.73 + 7.73) / 3 = 9.27 \text{ MHz}$$

Now, we are going to substitute these isotropic values to system {2} and to solve the system using the Excel file *Theta\_in\_tyr.xls*. I do not recommend to open this file within an Internet Explorer browser: although it could be done, some substantial functionality might be lost. So, save this file on your computer and then open it with Excel (remember to

allow macros when prompted). Input the isotropic hyperfine splitting constant values to cells B7 and C7 on the 'Input data' spreadsheet. The values 22.00 and 9.27 MHz should first be converted to Gauss: 7.88 G and 3.32 G (the conversion coefficients are on the spreadsheet). Having followed the instruction, we will get four solutions of system {2} (Fig. 4).

methylene protons {2}. Two of the four solutions are characterised by a common value of  $\rho_{C1}$  and two rotational conformations (two values of  $\theta$ ) symmetrical with respect to  $-30^\circ$ . The other two solutions are characterised by another value of  $\rho_{C1}$ , also common for both, and by two rotational conformations symmetrical with respect to  $60^\circ$ . Certainly, only one of the two possible values of  $\rho_{C1}$  is correct and

Fig. 4 shows that there are always four solutions of the system of two McConnell equations written for the two

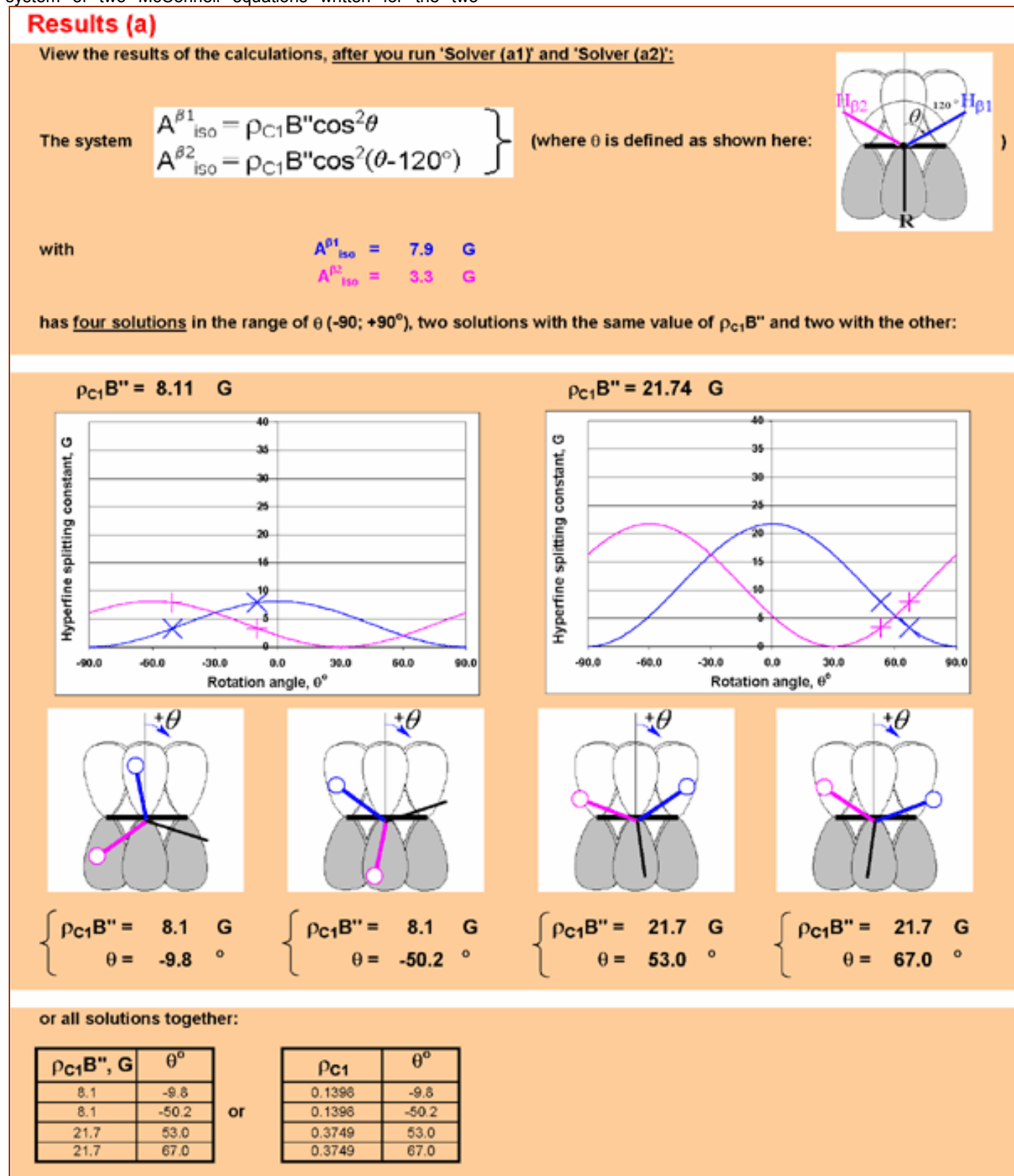


Fig. 4. The output result of the Excel file-calculator *Theta\_in\_tyr.xls* when the input parameters of the hyperfine splitting constants for the methylene protons were set as 22.00 MHz (7.88 G) and 9.27 MHz (3.32 G). There are four solutions (pairs of  $\theta$  and  $\rho_{C1}$ ) of system {2}.

corresponds to the spin density on atom C1 in the radical. Analysis of literature data on tyrosyl radicals shows that it must be the value of 0.375, and the value of 0.140 is too low to be true and therefore should be discarded. Thus, we are left with two possible values of rotational angle  $\theta$ , both for  $\rho_{C1} = 0.375$ :  $\theta = 53.0^\circ$  and  $\theta = 67.0^\circ$ . These two angles are equivalent from the EPR point of view. There is no way to say from the EPR spectrum alone, which of these two rotational angles is the characteristic of the radical observed.

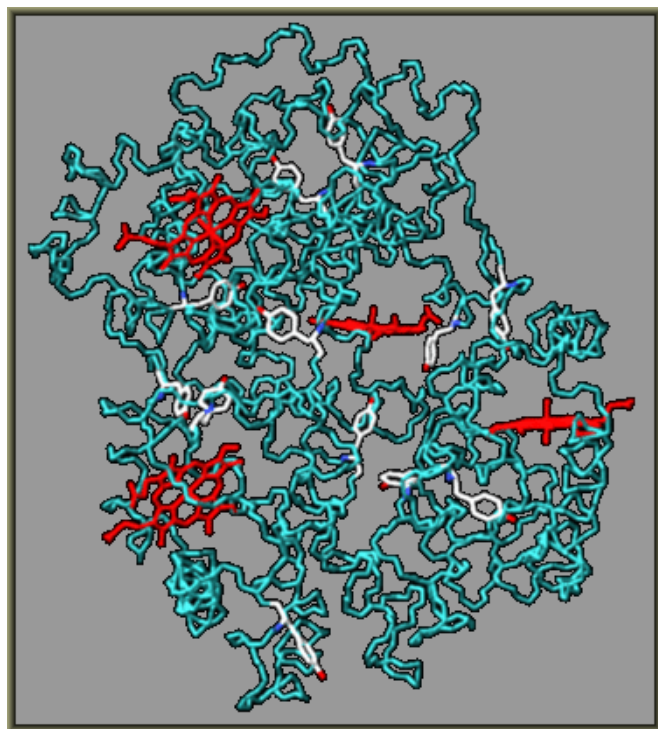
#### 4.1.2. How to find $\theta$ in different Tyr residues

Here we come to the point when our database should be used. We should find the Tyr residues in the human haemoglobin structure with rotational conformation of the ring close to either one or the other possible angle found by the EPR spectroscopy, i.e.  $53.0^\circ$  or  $67.0^\circ$ .

Human haemoglobin has twelve Tyr residues, three in each subunit (Fig. 5).

We can now go to 'Search', specify haemoglobin's PDB ID code (1HGB) in the pull-down menu and have the table of all twelve Tyr residues in this protein with individual values of angle  $\theta$ . By default, the data in the table are sorted by tyrosine number. You can sort the data also by chain (A, B, C and D in our example), by  $\theta$  or by the difference  $|\theta - \theta_{\text{target}}|$ . We will be particularly interested in the last option, having specified this target value of  $\theta$ , first, as  $53.0^\circ$  and then as  $67.0^\circ$ . When the data are sorted in this way, the residues with the most close rotational conformation to either  $53.0^\circ$  or  $67.0^\circ$  will be arranged descending from the top.

Chains A and C in haemoglobin are identical (the two  $\alpha$ -subunits), so are chains B and D (the  $\beta$ -subunits). However, homologous tyrosine residues not always have identical, or at least very close conformations: while angle  $\theta$  in  $\alpha$ Tyr140 has close values in A and C chains, and the same is true for  $\beta$ Tyr145, the rotational angles in other homologous residues are different by 9-13 $^\circ$ .



**Fig. 5.** Human haemoglobin with highlighted four haem groups and twelve tyrosine residues. Rotational conformation of the ring, defined by individual electrostatic environment, is different in different tyrosine residues. The figure was generated for the 1HGB coordinate file [Liddington, R., Derewenda, Z., Dodson, E., Hubbard, R., Dodson, G.: High resolution crystal structures and comparisons of T-state deoxyhaemoglobin and two liganded T-state haemoglobins: T(alpha-oxo)haemoglobin and T(met)haemoglobin. *J Mol Biol* 228 pp. 551 (1992)].

The speculation on the nature of this effect is out of the scope of this database description. Instead, we would average the values of  $\theta$  for homologous residues and sort the data by the difference between this averaged angle and the two target values. This could be easily done if we copy the table on the web page and paste it into a blank Excel spreadsheet. The result would be as follows:

Sorted by  $|\theta_{\text{average}} - \theta_{\text{target}}|$

| when $\theta_{\text{target}} = 53.0^\circ$ : |                           |  |
|--|---------------------------|--|
|  | $\theta_{\text{average}}$ | $ \theta_{\text{average}} - 53^\circ $ |
| $\alpha$ Tyr42                               | 56.5                      | 3.5                                    |
| $\beta$ Tyr130                               | 45.2                      | 7.8                                    |
| $\beta$ Tyr35                                | 44.2                      | 8.8                                    |
| $\beta$ Tyr145                               | 85.9                      | 32.9                                   |
| $\alpha$ Tyr24                               | -37.9                     | 90.9                                   |
| $\alpha$ Tyr140                              | -81.9                     | 134.9                                  |

| when $\theta_{\text{target}} = 67.0^\circ$ : |                           |  |
|--|---------------------------|--|
|  | $\theta_{\text{average}}$ | $ \theta_{\text{average}} - 67^\circ $ |
| $\alpha$ Tyr42                               | 56.5                      | 10.5                                   |
| $\beta$ Tyr145                               | 85.9                      | 18.9                                   |
| $\beta$ Tyr130                               | 45.2                      | 21.8                                   |
| $\beta$ Tyr35                                | 44.2                      | 22.8                                   |
| $\alpha$ Tyr24                               | -37.9                     | 104.9                                  |
| $\alpha$ Tyr140                              | -81.9                     | 148.9                                  |

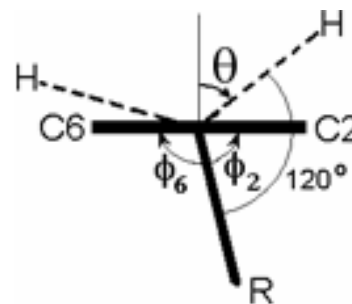
Thus, a formal use of our database allowed us to produce a short list of tyrosine residues in the human haemoglobin structure which are the most likely candidates to host the observed tyrosyl radical. The smallest difference  $|\theta_{\text{average}} - \theta_{\text{target}}|$  is found for Tyr42 in the  $\alpha$ -subunits when  $\theta_{\text{target}} = 53^\circ$ . Therefore, this tyrosine should be considered as the most probable site of the free radical location. Next probable in the list would be are Tyr130 and Tyr35 in the  $\beta$ -subunits if  $\theta_{\text{target}} = 53^\circ$  and Tyr42 in the  $\alpha$ -subunits if  $\theta_{\text{target}} = 67^\circ$ .

## 5. How to submit an entry to the database

If you happened to measure, at least once in your work before, a phenol ring rotation angle in a tyrosine in a protein, you can submit the value to the database.

You will have to register (follow the link on the [top of this document](#)) in order to be able to submit data to this database. Once your registration is confirmed by an e-mail message, you can log in (the link on the [top of this document](#)) and select from the three possible options: update your personal data, add records to the database, edit your previous record(s) to the database.

Please note that you are not expected to submit actual value of  $\theta$ . Angle  $\theta$  cannot be measured directly because protons are not resolved in the crystal structure. Instead, we have to assume that each C $\beta$ -H $\beta$  bond is forming a  $120^\circ$  angle with the R-C $\beta$  bond, when using view A defined in Fig. 1, and, of course, the angle between C $\beta$ -H $\beta_1$  and C $\beta$ -H $\beta_2$  is also  $120^\circ$  (Fig. 6.) Therefore, we have to measure the dihedral angle between the ring plane and the R-C $\beta$ -C $\alpha$  plane,



**Fig. 6.** The ring rotation angle  $\theta$  can be found from directly measurable dihedral angles  $\phi_2$  and  $\phi_6$ . From this diagram:  $\theta^\circ = 90^\circ - (120^\circ - \phi_2) = \phi_2 - 30^\circ$ . Angle  $\phi_2$  is only approximately equals to  $(180^\circ - \phi_6)$  since the ring is never ideally flat in the real situations, so we use an average value instead of  $\phi_2$ :  $\theta = ((180^\circ - \phi_6) - \phi_2)/2 - 30^\circ$  (note that  $\phi_2$  and  $\phi_6$  have opposite sign).

i.e. either angle  $\phi_2$  or angle  $\phi_6$  (Fig. 6). Ideally, the sum of these two angles should be equal to  $180^\circ$ . In reality it is never the case since the six carbon atoms of the ring never form a perfect plane. Therefore, the server of our database takes the values of both angle  $\phi_2$  and  $\phi_6$  as an input, calculates an average value for two angles,  $\phi_2$  and  $(180^\circ - \phi_6)$ , and uses this average value for calculation of angle  $\theta$  (see Fig. 6).

### 5.1. How to measure angles $\phi_2$ and $\phi_6$

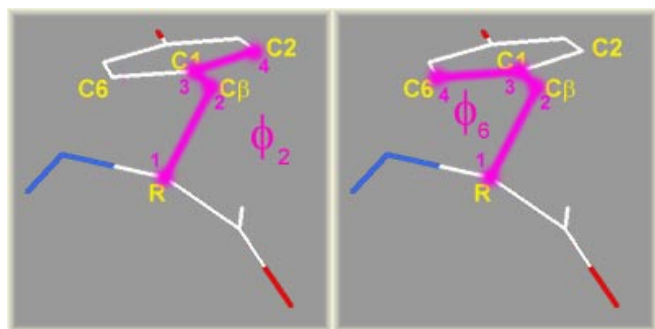
How can we measure  $\phi_2$  and  $\phi_6$ ? [Swiss PdbViewer](#) is a free program that allows viewing and analysing 3-dimensional structure of biomolecules. It has a tool for measuring dihedral angles. Once you have a tyrosine zoomed-in on your screen, you can measure angles  $\phi_2$  and  $\phi_6$ . First, press Ctrl then click on this button :



The appearance of this button is probably the same in different version of PdbViewer; this one is as in version 3.7

Then click consecutively on atoms R, C $\beta$ , C1 and C2 for  $\phi_2$  or on atoms R, C $\beta$ , C1 and C6 for  $\phi_6$  (Fig. 7, note that atom nomenclature will not be displayed).

The value of the dihedral angle will be displayed on the fourth click. Having measured  $\phi_2$  and  $\phi_6$  for a tyrosine residue, you can go straight to the submission page and submit these two values; angle  $\theta$  will be calculated automatically and added to the database.



**Fig. 7.** The sequence of atoms to click on when measuring the values of the angles  $\phi_2$  and  $\phi_6$  with [Swiss PdbViewer](#).

### 6. What's next? How this database will be developed

This database was created in an attempt to systematise my collection of rather fragmented data on angle  $\theta$  in different tyrosine residues of different proteins. So I decided to put whatever I had at the time on-line and to make it possible for the others to add new data. The limitation of the database in such format is in the fact that it contains only the records of the angles that happened to be submitted. The database is not comprehensive at the moment. When launched, the database contained records for 191 tyrosine residues in 27 proteins, and there is still a number of cases when not all tyrosines of a particular protein are present in the database.

I am very determined to change this. I plan to create a program that would append a structure file from the Protein Databank, would calculate angle  $\theta$  for all tyrosine residues using the atoms coordinates and would add the result to the database. This would make the database completely comprehensive: for every protein structure present in PDB, angle  $\theta$  could be found and ranked for all tyrosine residues. When this task is accomplished, the database will become a very powerful tool in assigning a tyrosyl radical to a particular residue in proteins.

There are also other things which form the following 'to do' list:

- To make available the option of averaging of angle  $\theta$  in homologous tyrosine residues in identical chains (within the same structure).
- To make available the option of viewing (and averaging if necessary) the data on angle  $\theta$  in the same tyrosine residues present in different structure files (by 'different structure files' I mean either the same protein reported at different times or two or more modified structures of the same protein, e.g. by mutation, ligation, pH etc.).
- To make available the option of viewing the data on angle  $\theta$  in conserved tyrosine residues in different (related) proteins.
- To replace all Excel driven fragments of this web site with JavaScript.
- To set up a discussion group.
- To add material about computer simulation of the tyrosyl radical EPR spectra (once my recently submitted paper is out).
- To make the appearance of this web site browser insensitive (originally it was composed for best viewing with Internet Explorer).

### 7. About this database and Contact

The Phenol ring rotation angle database for tyrosine residues in different proteins is a free resource created by Dimitri Svistunenko, University of Essex. References to this database in publications will be appreciated. The use of images of tyrosine conformation generated for a pre-set value of rotational angle  $\theta$  (Fig. 2 in the interactive mode) is allowed in publications with a reference to this database. The use of the Excel file-calculator *theta\_in\_tyr.xls* in any published work is allowed with a reference to this database.

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