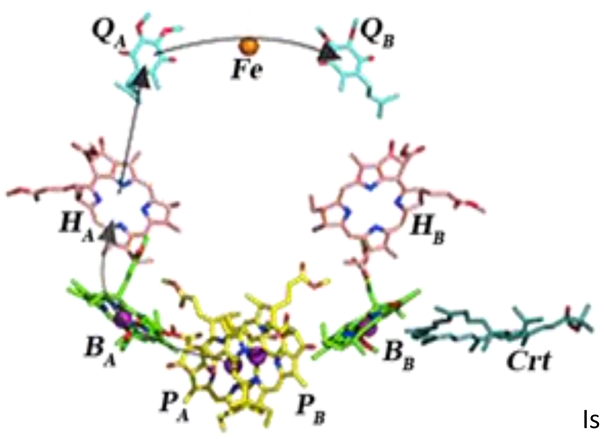
Identification of hidden parameters of conformational states during photoexcitation of protein macromolecules

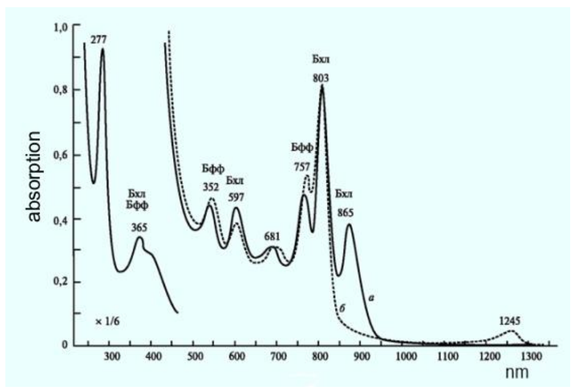
Yurii Barabash

Department of Physics of Biological Systems, Institute of Physics, Natl. Acad. of Sci. of Ukraine. Prospect Nauky, 46, UA-03028, Kyiv, Ukraine. E-mail ybar@ukr.net

INTRODUCTION

The aim of the work is to develop software based on the method of category for identifying various redox conformational states of biological macromolecules. Features of conformational states affect the functioning of biomacromolecules, it is difficult to measure in the experiment, being the hidden parameters of macromolecules. Such parameters are found by solving the global optimization problem, which is **relevant** in many cases. **As an object**, isolated reaction centers of ***Rhodobacter sphaeroides***, the structure of which is well studied, were used. **The methods** of optical absorption spectroscopy and procedures for computer analysis of experimental data were used. For excitation of the RC, light with a wavelength of λ=865 nm, an intensity of 0.5÷7 mW/cm^2, was used for 100 s (external influence). This caused a decrease in the absorption of the RC suspension at the wavelength of light λ = 865 nm, which is associated with the photo transfer of the electron from the donor to the secondary acceptor RC (main reaction). The normalized kinetics of electron photo transfer both at the stage of the RC illumination and at the RC relaxation stage (after the end of RC illumination) corresponded to the sum of three (N=3) exponential functions that had different negative decrement values.

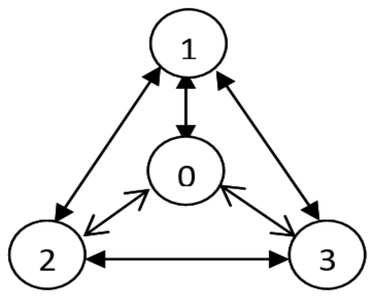




Is the spectrum absorption of the RC in the dark (a).

Is the spectrum of the illuminated RC (b).

Methods of optical absorption spectroscopy and procedures of computer analysis of experimental data were used. It was found that the absorption kinetics of isolated RCs both at the stage of illumination and at the stage of relaxation (after the end of RC illumination) corresponds to the sum of three (N = 3) different exponential functions and the material balance equation. This corresponds to one of four solutions of the system of three conventional differential equations of electron transfer RC. The solutions of the system describe the behavior of four redox conformational states of the RC. One state is measurable and three states are hidden. The task of this work is to determine the reaction rate constants for differential equations in different modes of RC photoexcitation. The program used the Taboo Search (TS) algorithm, which is an "intelligent" method for minimizing multi-parameter functions. TS algorithm based on the prohibition of searching in certain ranges of values of the required parameters. The algorithm uses a modification of the "local search", which starts its work with some initial configuration of the required parameters and tries to find the best solution. This results in a new configuration, and the search process is repeated using this configuration as the initial one. He creates a table from the previously performed steps in the search for the minimum of the objective function and stores it in memory which is continuously updated. Using previously accumulated information, new search steps can be prohibited or allowed depending on the success of the search in the previous step.. d_i The difference between the experimental and calculated parameter values of the exponential functions was used as the objective function (Δx). It is defined as:



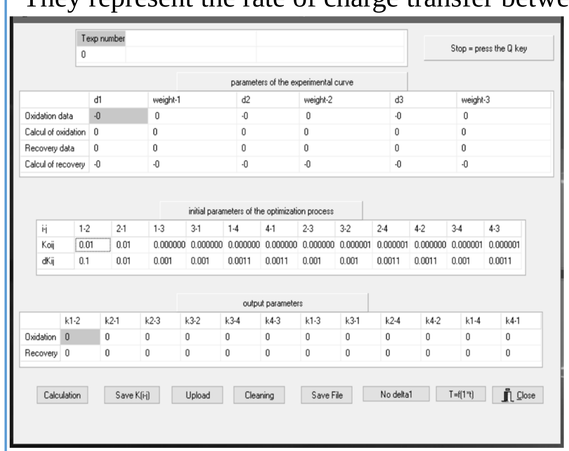
Kinetic scheme of electron transfers in RC

$$\Delta_x = \sum \frac{(A_i^0 - A_i^x)^2}{(A_i^0)^2} + \frac{(d_i^0 - d_i^x)^2}{(d_i^0)^2} \quad i = 1,2,3$$

They represent the rate of charge transfer between the subsystems of the RC.

$$dX_0/dt = (k_{01}+k_{02}+k_{03})X_0 + k_{10}X_1 + k_{20}X_2 + k_{30}X_3$$
$$dX_1/dt = k_{01}X_0 (k_{10}+k_{12}+k_{13})X_1 + k_{21}X_2 + k_{31}X_3$$
$$dX_2/dt = k_{02}X_0+k_{12}X_1 -(k_{20}+k_{21}+k_{23})X_2+k_{32}X_3$$
$$X_0+X_1+X_2+X_3=1$$

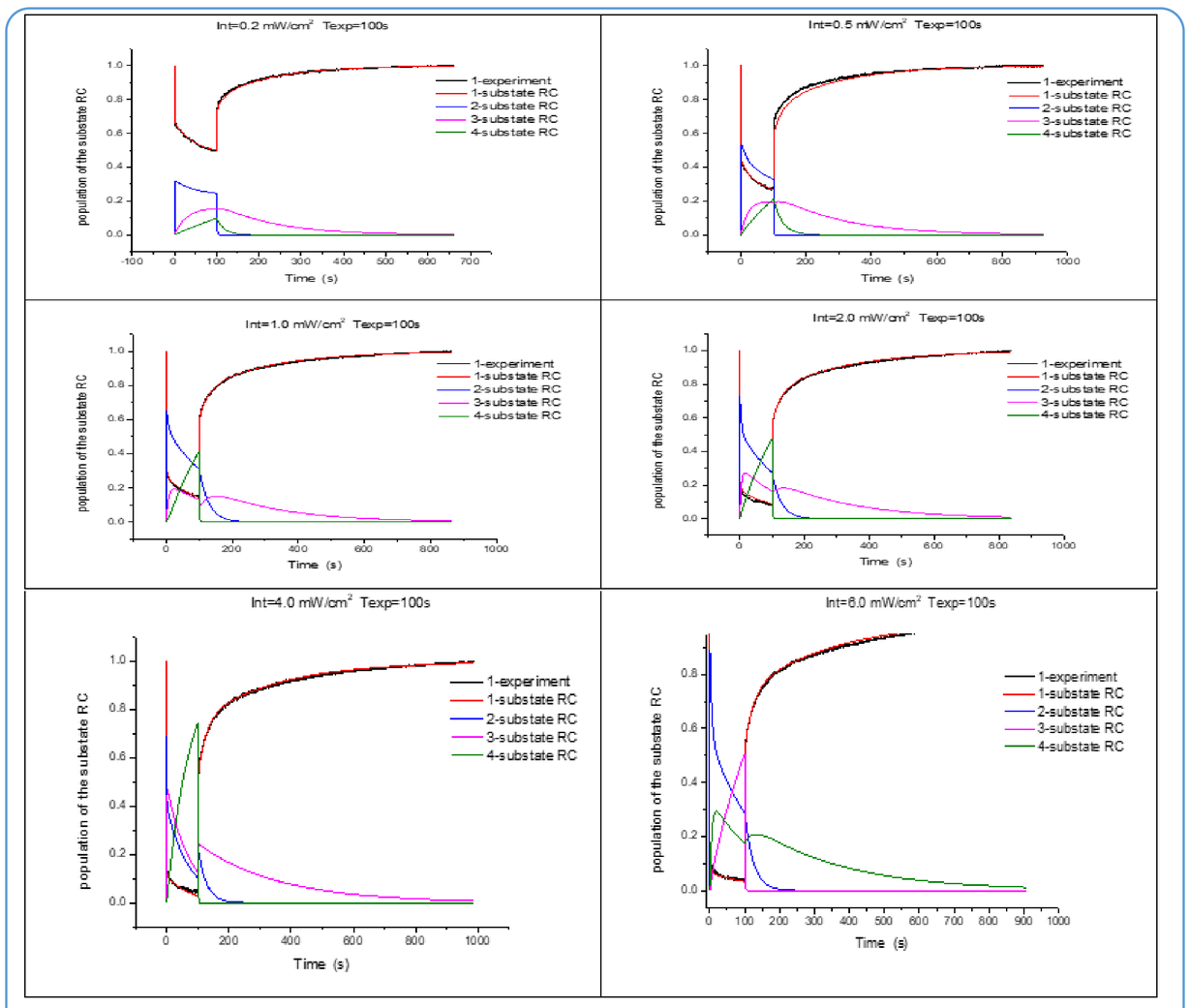
The arrows on the diagram show the electron-conformational interaction of the RC complex. The rate constants (kij) of the differential balance equations (12 pieces) in the figure are indicated by arrows. They represent the rate of charge transfer between the subsystems of the RC.



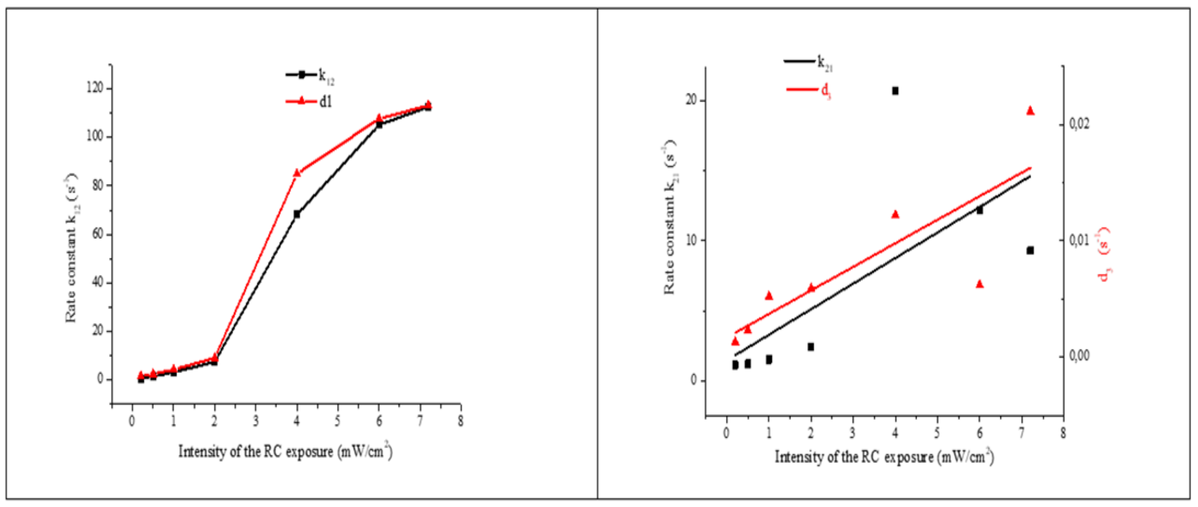
Where Xj(t) is the time dependence of the probability density of finding an electron in the j-state of RC (population of the states of the RC), j = 1,2,3,4. The initial conditions of the populations of the states of RC during exposure are: $X_0(0) = 1$, $X_1(0) = 0$, $X_2(0) = 0$, $X_3(0) = 0$. The initial conditions of the populations of the states of the RC after the exposure of RC are equal to the populations of the states of RC at the end of the exposure: $X_0^{rel}(0) = X_0(Texp)$, $X_1^{rel}(0) = X_1(Texp)$, $X_2^{rel}(0) = X_2(Texp)$, $X_3^{rel}(0) = X_3(Texp)$ The solution of the system is:

$$X_i(t)=\sum C_{j,i}\exp(-d_i t) \quad X_3(t)=1-\sum X_j(t) \qquad i=1,2,3;\ j=0,1,2$$

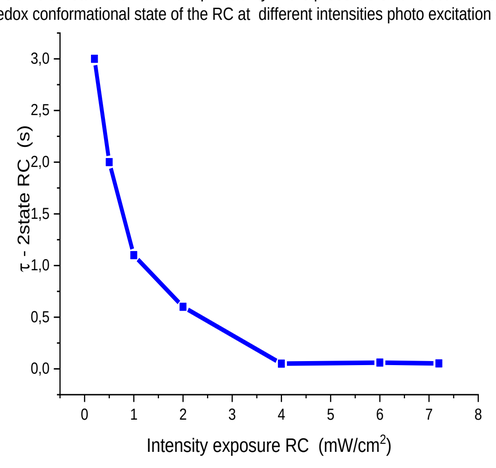
di - are equal to the eigenvalues of the matrix of constants of differential equations; *Cji* - are constants defined by the coordinates of the eigenvectors of the matrix of differential equations and the initial conditions of the states of RC. The eigenvalues of the matrixes of coefficients of differential equations for photoexcitation and relaxation processes RC should not be equal to zero (***di***≠0). his imposed a restriction on the initial values of the constants of differential equations. **As a result** a program was developed that uses the parameters of the experimental kinetics of the cyclic electron transfer of the RC, **determines** the values of the reaction rate constants for four differential equations, the peculiarities of the kinetics of the probability of finding an electron in four redox conformational states of the RC.



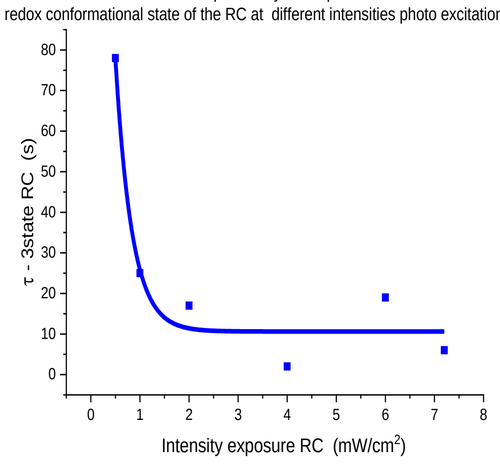
The probability density of the presence of an electron in various redox conformational states of the RC. The coincidence of the calculated and experimental dependences of the presence of an electron in the ground state (0) at the stage of illumination of the reaction center and the stage of relaxation of the reaction center after illumination is shown.



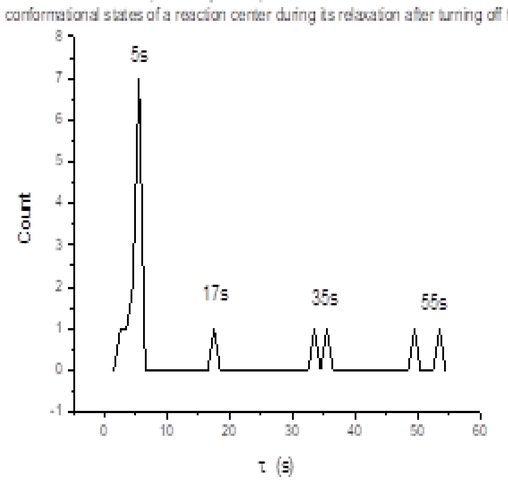
The time to reach the maximum probability of the presence of an electron in the 2nd redox conformational state of the RC at different intensities photo excitation of the RC



The time to reach the maximum probability of the presence of an electron in the 3rd redox conformational state of the RC at different intensities photo excitation the RC



Minimax values of the probability of the presence of an electron in various redox conformational states of a reaction center during its relaxation after turning off the light



This may be due to an increase in the energy of reorganization as the result of a change in the polarization of the protein molecules of the electron transfer chain during its cyclic transfer.

It has concluded that the RC correspond to a system of four electron-conformational states. The features of the time dependence of the probability of finding an electron in conformational states characterize the space-time motions of the RC.