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Spectral manifestations of bacteriochlorophyll hydrogen bonding with various polar solvents

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Received December 28, 2023
Revised February 02, 2024
Accepted March 05, 2024

Interaction of bacteriochlorophyll e with various polar solvents such as water, methanol, ethanol and isopropanol has been studied. Calculations were performed using the Gaussian software package and the 6-31G (d) basis to determine the structure of bacteriochlorophyll and the properties of its hydrogen bonds. Results confirm the importance of hydrogen bonds in bacteriochlorophyll interaction with polar solvents. These solvents are most often used for extraction of bacteriochlorophylls from bacterial cells, so this work is useful for developing methods for quantitative determination of bacteriochlorophyll e in bacterial cells or in bodies of water.

Keywords: bacteriochlorophyll, polar solvents, molecular modeling, infrared spectrum, density functional theory, hydrogen bonds.

DOI: 10.61011/EOS.2024.04.58876.36-24

Introduction

Bacteriochlorophylls are a group of photosynthetic tetrapyrrole pigments that are capable of absorbing light in the near infrared (IR) spectral region, which is beyond the absorption range of common chlorophyll [1]. This absorption capability is especially useful in aquatic environments where longer wavelengths of light penetrate deeper. In contrast to oxygenic photosynthesis in plants [1], bacterial photosynthesis with bacteriochlorophyll (BChl) does not normally produce oxygen as a byproduct.

Several types of BChl designated by the letters a through g are known. They have slightly different structures and are found in different groups of bacteria [2]. These adaptations lend a unique absorption spectrum to each type of BChl, allowing photosynthetic bacteria to occupy a range of ecological niches with varying light conditions. The present study is focused on BChl e and its interaction with polar solvents (water, methanol, ethanol, and isopropanol).

Bacteriochlorophyll e (Fig. 1) efficiently absorbs light with a wavelength of approximately 500−550 nm. This distinguishes it from other bacteriochlorophylls, such as BChl c and BChl b, that absorb light in different regions of the spectrum with wavelengths of about 740−760 nm and 808−866 nm, respectively [1]. In addition, bacteriochlorophyll e features certain structural differences. It contains a methyl group [4], which makes it more hydrophilic than other types of bacteriochlorophyll. This affects its solubility and interaction with other molecules in a cell.

Chlorosomes of green sulfur bacteria (GSBs) are large photosynthetic antenna complexes containing self-assembling structures of bacteriochlorophyll c, d, or e (Fig. 2) [5]. Unlike other light-collecting antenna structures, the primary antenna pigments (BChl c, d, or e) form aggregates that do not require a protein scaffold [6]. This is made possible by structural modifications that are found in these BChls and are lacking in other naturally occurring chlorophyll derivatives. These properties enable the formation of very large and efficient antenna complexes [7,8] that ensure phototrophic growth at extremely low light intensities [7]. One photosynthetic GSB unit (chlorosome) may contain 900–4500 molecules of BChl d or e and 80−250 molecules of BChl a [9].

Absorption spectra of BChl d and e both in samples of natural water with microorganisms and in extracts in organic solvents are used to estimate the concentration of GSBs in water bodies [10]. The most convenient methods for quantitative assessment are those based on the use of

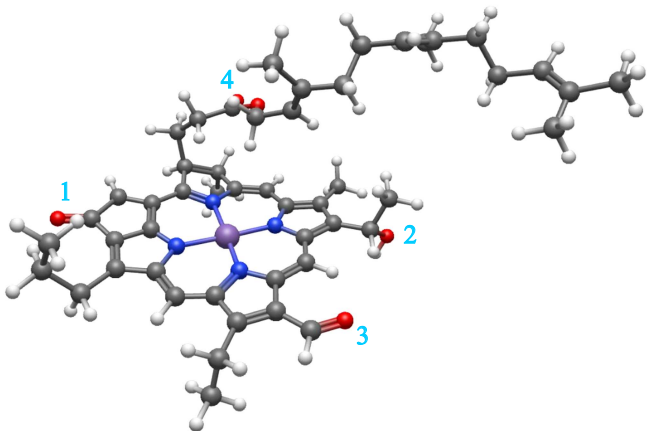

Figure 1. Calculated structure of bacteriochlorophyll e (numbers indicate the sites where hydrogen bonds based on the OH group may be formed).

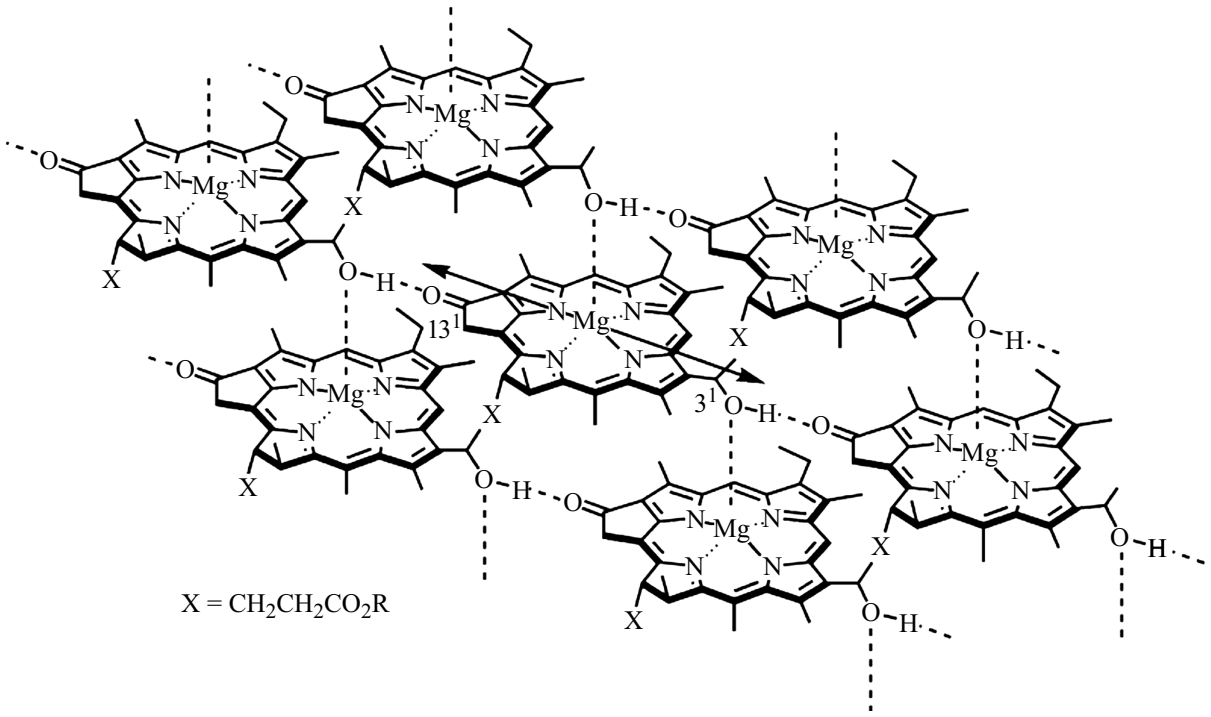

Figure 2. Structure of the supramolecular complex of bacteriochlorophyll [6].

absorption characteristics of pigments. The concentration of BChl in certain natural water samples may be estimated from the fluorescence spectra of their extracts [11].

Thus, this line of research is critically important for monitoring of stratified water bodies (including relict ones in the Arctic region) with sulfide anoxia, which may occur naturally or as a result of anthropogenic pollution.

Bacteriochlorophylls are unstable against light, acids, and oxidants. They undergo allomerization readily In polar solvents (e.g., in methanol). The release of the monomeric pigment form in this process induces a change in the spectral properties of pigment molecules. Experimental studies of the optical absorption spectra of bacteriochlorophyll with various polar solvents (Fig. 3) revealed that they differ significantly in intensity [9], providing evidence of the effects of hydrogen bonding in these multicomponent mixtures. It is assumed that the hydrogen bonding properties become progressively more prominent in the following sequence of polar solvents: acetone–isopropanol–ethanol–methanol. Thus, hydrogen bonds play an important role in the processes of allomerization and variation of the spectral properties of bacteriochlorophyll in polar solvents. A deeper understanding of these bonds and their influence may facilitate the application of bacteriochlorophylls in various fields of science and technology.

Computer modeling of IR spectra

Modeling of the structure and calculations of the spectra of molecules and their complexes were carried out on the basis of density functional theory (DFT) [12] using the B3LYP functional and the 6-31G (d) basis set [13]. All molecular modeling procedures (including optimization of molecular structures and calculation of IR spectra) were performed in the Gaussian software package [14], which is used widely for molecular modeling in various fields of computational physics and chemistry, with the use of the Avogadro editor and visualizer of molecular structures [15] and proprietary IR spectra visualization software that reproduces an IR spectrum based on numerical values determined in Gaussian.

Frequency scaling, which is used often by research groups around the world [16,17], was performed to obtain a closer agreement between measured and calculated spectra. The scaling factors were as follows: 0.97 for the $0-2000\,\mathrm{cm}^{-1}$ frequency range and 0.95 for the $2000-4000\,\mathrm{cm}^{-1}$ frequency range.

Molecular modeling methods were used to calculate the structures of bacteriochlorophyll e (Fig. 1); its polar solvents (water, methanol, ethanol, and isopropanol); and complexes of bacteriochlorophyll e with these solvents: with water (Fig. 4, *a*), methanol (Fig. 4, *b*), ethanol (Fig. 4, *c*), and isopropanol (Fig. 4, *d*). The OH group was considered as a possible addition to BChl e in solvents (water — $3658\,\mathrm{cm}^{-1}$, methanol — $3566\,\mathrm{cm}^{-1}$, ethanol — $3561\,\mathrm{cm}^{-1}$, and isopropanol — $3551\,\mathrm{cm}^{-1}$). The degree of complexation of bacteriochlorophyll with polar solvents was assessed by monitoring the changes in these frequencies. Indices 1–4 in Fig. 1 mark the sites of BChl binding to polar solvents.

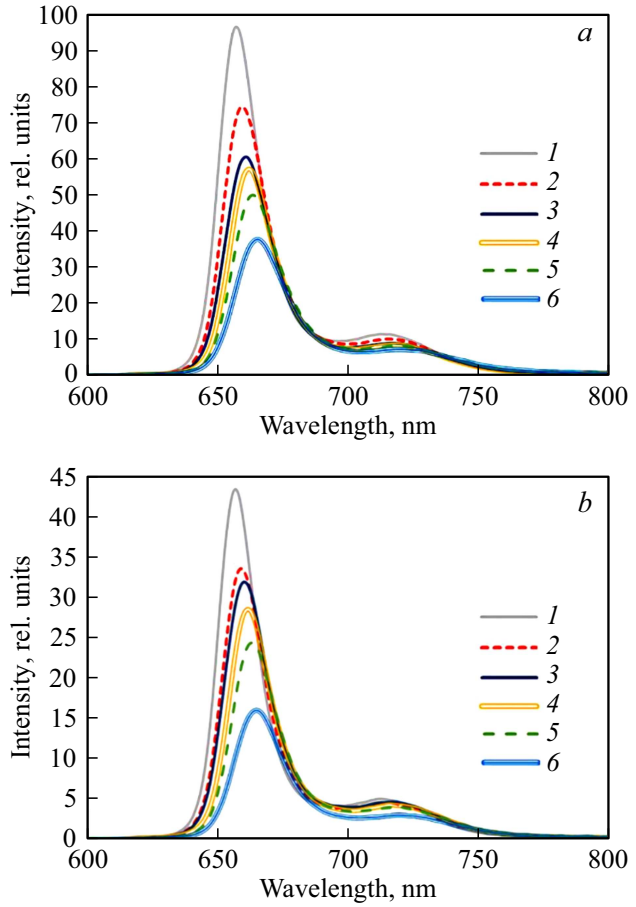

Figure 3. Fluorescence spectra taken from [9]: bacteriochlorophyll d (*a*) and bacteriochlorophyll e (*b*) in acetone (*1*), acetone–ethanol (7:2) (*2*), acetone–methanol (7:2) (*3*), isopropanol (*4*), ethanol (*5*), and methanol (*6*) (at an excitation wavelength of 425 nm).

The lengths of hydrogen bonds O · · · H-O in nanometers are also indicated next to these indices.

Figure 5 shows the IR spectra of molecular complexes of bacteriochlorophyll e with polar solvents. The peaks of bond vibration frequencies are marked with dots. It is worth noting that four regions are distinguished clearly in the calculated IR spectra: the region of frequencies up to 1800 cm^{-1} corresponds to the deformation and valence vibrations of C-C, C-O, C-N, and N-Mg bonds; the peaks within the 2700–3150 cm^{-1} range correspond to the asymmetric and symmetric vibrations of C-H bonds of alkanes; the spectral peaks of hydrogen bonding between oxygen and the OH group of polar solvents of bacteriochlorophyll e are found in the region from 3350 to 3520 cm^{-1}; and the frequency of 3555 cm^{-1} corresponds to the vibrations of O-H bonds.

Figure 6 presents a more detailed view of the high-frequency region of the IR spectrum of hydrogen interaction between BChl e and polar solvents. The frequencies of vibration peaks are indicated above the dots, and the peaks themselves are numbered in accordance with the numbering of attachment sites shown in Fig. 1.

Assessment of hydrogen bonds

The strength of formed hydrogen bonds was assessed by the following parameters: the hydrogen bridge length and the frequency shift of valence vibrations of H-bonds in the IR spectrum of the molecular complex relative to the IR spectrum of individual molecules.

The following hydrogen bond parameters are listed in the table: bond type, initial bond length, O · · · H-O hydrogen bridge length, spectral line peak intensity, and frequency shift $\Delta\nu$ of valence vibrations of bonds in the IR spectrum of the molecular complex relative to the IR spectrum of individual molecules. This shift is needed to calculate the bond energy in accordance with the empirical Iogansen formula [18]:

$$-\Delta H = 0.3\sqrt{\Delta\nu - 40}.$$

The calculated parameters of hydrogen bonds between BChl e molecules and polar solvents are presented in the table. Classification [19] was used as a basis for assessing the strength of hydrogen bonds. According to this classification, bonds with energies of 14.34–28.65 kkal/mol and hydrogen bridge lengths of 2.2–2.5 Å are strong. Moderate-strength bonds have energies ranging from 3.82 to 14.43 kkal/mol and hydrogen bridge lengths of 2.5–3.2 Å. Weak bonds are characterized by energies below 2.87 kkal/mol and hydrogen bridge lengths of 3.2–4.0 Å. According to this classification, the bonds listed in the table are of a moderate strength.

The interaction sites (Fig. 1) should be taken into account when one performs a comparative analysis of IR spectra. One exception to this rule is bond number 3 of isopropanol: the size of isopropanol molecules, which are significantly larger than the molecules of other solvents, makes it impossible to form a sufficiently stable bond at site 3. With this taken into account, one can deduce from Fig. 6 and the table that the frequency peaks for attachment sites 1–4 shift to the left and the shift increases gradually in the following sequence of solvents: water–methanol–ethanol–isopropanol. This is also evidenced by shorter lengths of hydrogen bonds and hydrogen bridges and increasing intensities of hydrogen bonds.

Conclusion

The structure of BChl e and its complexes with water, methanol, ethanol, and isopropanol was determined and optimized by examining the interaction of bacteriochlorophyll e with polar solvents. The IR spectra of these structures were also calculated. These spectra suggest that the hydrogen bonding properties become progressively more prominent in the following sequence of polar solvents: water–methanol–ethanol–isopropanol.

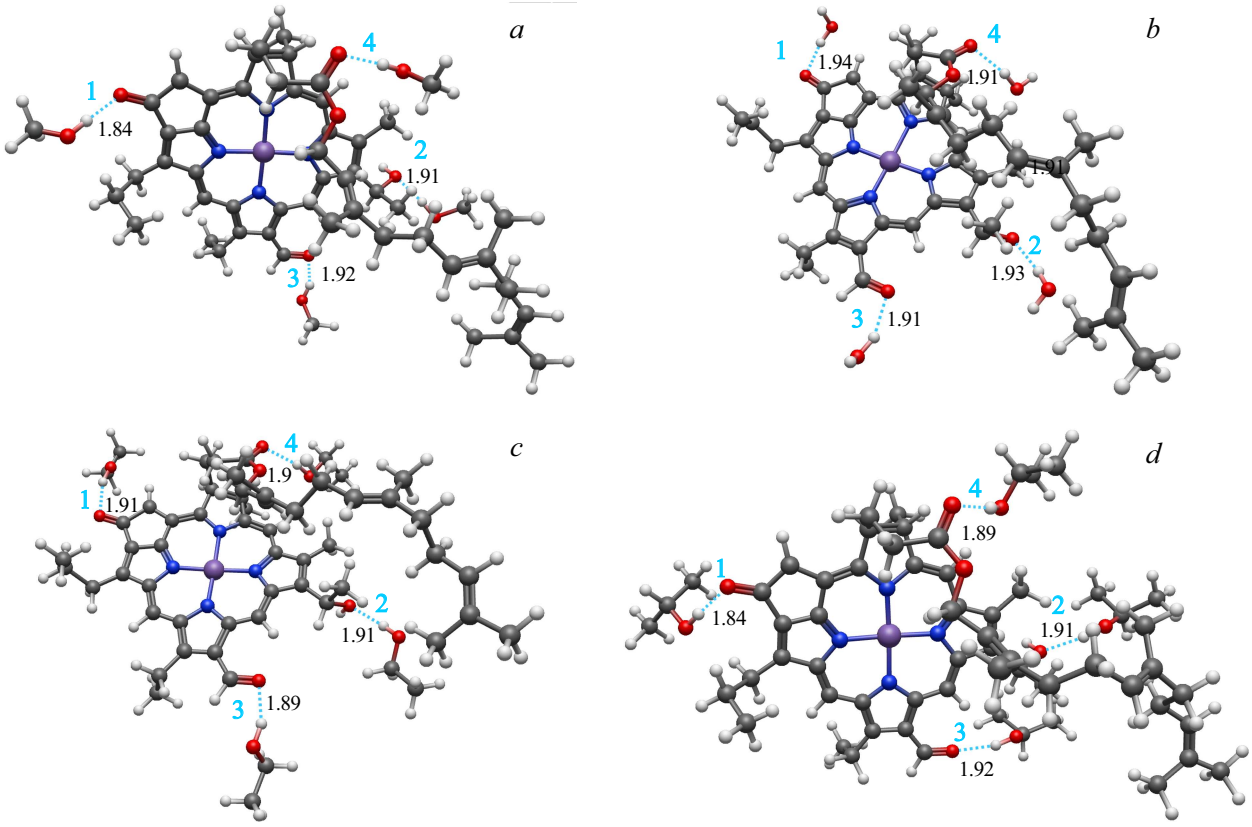

Figure 4. Calculated structures of BChl e with water (*a*), methanol (*b*), ethanol (*c*), and isopropanol (*d*). Hydrogen, carbon, oxygen, nitrogen, and magnesium atoms are colored gray, dark gray, red, blue, and purple, respectively.

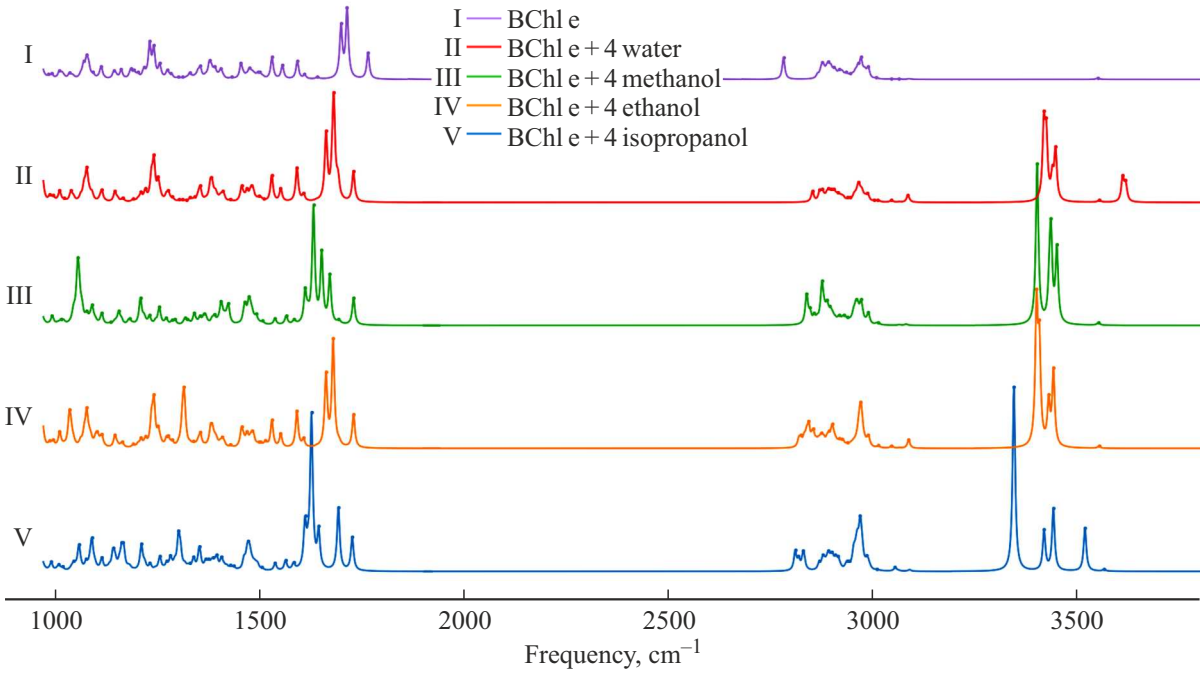

Figure 5. Infrared spectra of bacteriochlorophyll e (purple I) and complexes of bacteriochlorophyll e with various solvents: blue V — isopropanol, yellow IV — ethanol, green III — methanol, and red II — water.

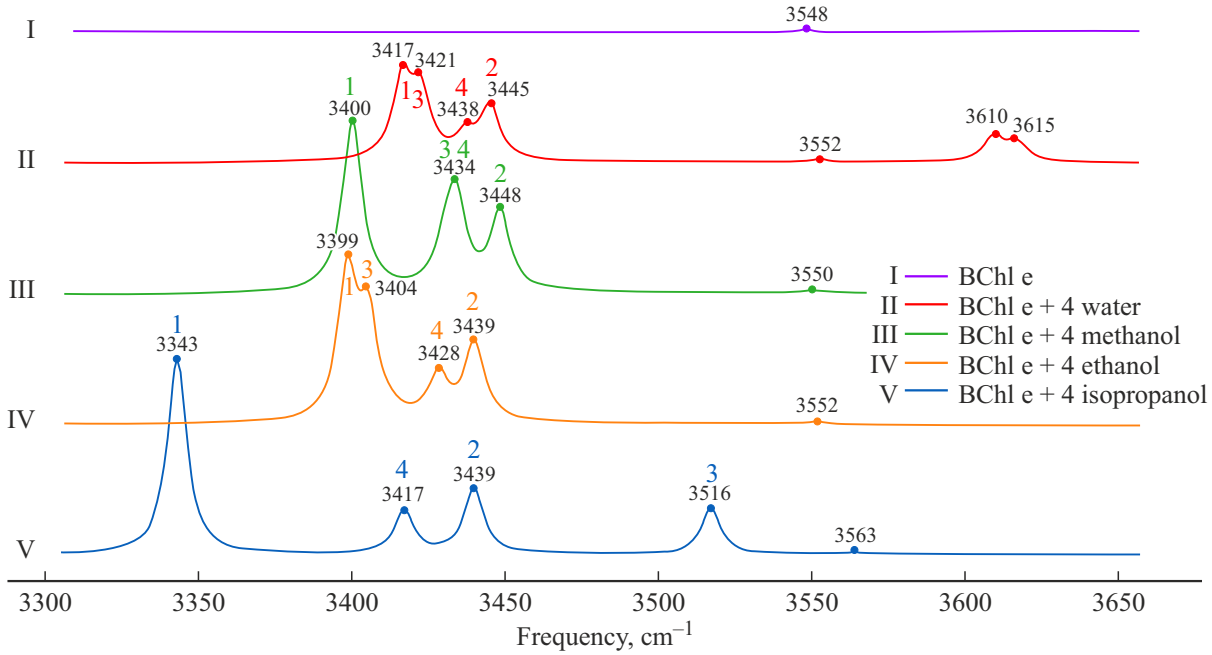

Figure 6. High-frequency region of the IR spectra of bacteriochlorophyll e (purple I) and complexes of bacteriochlorophyll e with various solvents: blue V — isopropanol, yellow IV — ethanol, green III — methanol, and red II — water.

Calculated parameters of hydrogen bonds

Bond number	Bond type	Bond length, Å	Hydrogen bridge length, Å	Frequency, cm^{-1}	Frequency shift $\Delta\nu$, cm^{-1}	Bond energy, ΔH, kkal/mol	Intensity, km/mol
Bacteriochlorophyll e with four water molecules $E = -3992.5$ a. u.							
1	O · · · H-O	1.94	2.88	3417	241	4.25	676
2	O · · · H-O	1.93	2.89	3445	212	3.94	467
3	O · · · H-O	1.91	2.83	3421	235	4.19	558
4	O · · · H-O	1.91	2.84	3438	220	4.03	204
Bacteriochlorophyll e with four methanol molecules $E = -4149.7$ a. u.							
1	O · · · H-O	1.84	2.82	3400	165	3.36	1514
2	O · · · H-O	1.91	2.87	3448	117	2.64	688
3	O · · · H-O	1.92	2.82	3434	131	2.87	777
4	O · · · H-O	1.90	2.88	3431	135	2.93	320
Bacteriochlorophyll e with four ethanol molecules $E = -4307.05$ a. u.							
1	O · · · H-O	1.91	2.86	3399	162	3.32	1275
2	O · · · H-O	1.91	2.86	3439	121	2.70	702
3	O · · · H-O	1.89	2.80	3404	155	3.22	875
4	O · · · H-O	1.90	2.83	3428	133	2.89	393
Bacteriochlorophyll e with four isopropanol molecules $E = -4464.33$ a. u.							
1	O · · · H-O	1.84	2.82	3343	208	3.88	1736
2	O · · · H-O	1.91	2.87	3439	111	2.53	581
3	O · · · H-O	1.92	2.87	3516	34	0.00	413
4	O · · · H-O	1.89	2.83	3417	134	2.91	413

Conflict of interest

The authors declare that they have no conflict of interest.

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Translated by D.Safin