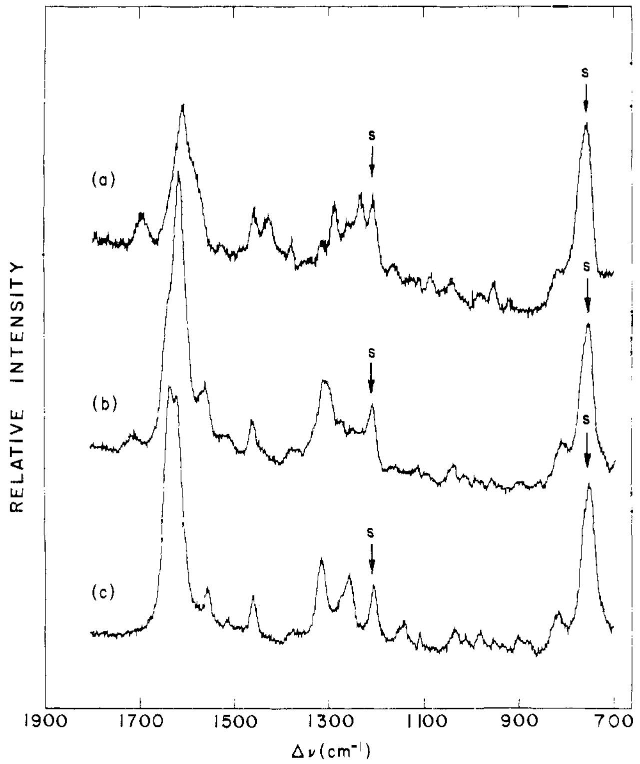

Figure 2. Resonance Raman spectra of **1** in chloroform: (a) neutral; (b) cation I; (c) cation II. Solvent lines are indicated by arrows.

temperature, despite the very low fluorescence quantum yield reported.[17] The Raman spectra of the solutions can be observed on the blue side of the fluorescence bands. Though the spectra are uncorrected for the instrumental sensitivity, some qualitative conclusions may be drawn. The neutral chloroform solution of **1** shows a broad fluorescence band with a maximum at ~704 nm which is split into two maxima (688 and 769 nm) upon shaking with 1 N HCl (cation I). Cation II has a maximum fluorescence at 738 nm, although a shoulder is still present at ~690 nm.[18]

It may therefore be concluded from the experimental evidence presented that different protonated species are present in media of different acidities.

The protonation effects are reversible. By shaking a solution containing cations I or II with 1 N aqueous NaOH, the original "neutral" absorption, fluorescence and RR spectra are recovered. This proves that we are dealing here with a pure protonation–protonation effect, and that the species identified as cations I and II do not correspond to degradation products of **1**.

A complete RR study of **1**, covering polarization and excitation profile measurements and a theoretical conformational analysis, is still in progress.

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On the Origin of the Red Shift of the Absorption Spectra of Aggregated Chlorophylls

Sir:

The reaction center of photosystem I in green plants (P700) is believed to involve a special dimer of a monohydrated chlorophyll *a* (Chl $a\cdot H_2O)_2$ (for recent reviews see ref 1–3 and references cited therein). Understanding the electronic potential surfaces of this dimer is essential for the elucidation of the dynamics and mechanism of the first step of the photosynthetic process. Direct information about the electronic states of chlorophyll polymers come from the absorption spectra of different types of aggregated chlorphylls. While the monomeric hydrated Chl *a* absorbs at ~665 nm (15 037 cm^{-1}),[2] the photoreactive dimer (P700) and related in vitro prepared dimers absorb at ~700 nm (14 286 cm^{-1}). Crystals and some types of surface layers absorb near 735 nm (13 605 cm^{-1}).[4]

The nature of the absorption red shift observed in Chl *a* aggregates has not been resolved despite many studies.[2-7] It was realized in recent years[2-4,7] that the red shift cannot be due to simple exciton interactions, because these account for only about 300 cm^{-1} of the observed shift. Shipman et al.[3b] and Fong and Wassam[2] attributed the large difference between the calculated exciton shift and the observed shift to environmental effects (dipole–dipole interactions with the solvent and/or other chlorophyll molecules). Kartky and Dunitz[4] suggested that the major inadequacy of the exciton model is the breakdown of the zero overlap approximation. Unfortunately, these explanations were not verified by calculations or by direct experiments and the origin of the dimer red shift remains obscure.

As long as the structure of P700 is not known, it is impossible to determine definitely the origin of its red shift. However, now it is possible to calculate the absorption spectrum of a chlorophyll dimer in a nonpolar solvent and to find out whether it can account for the red shift of P700. Here we present such quantum mechanical calculation on a chlorophyll dimer, where the two molecules are fixed in the relative geometry found in crystals of chlorophyll derivatives and surrounded by a nonpolar solvent. *This calculation gives a red shift almost as great as that found in P700.* The same type of calculation also reproduces the experimental red shift in a well-defined system of a crystal of a chlorophyll derivative. Our calculations employ the QCFF/PI method[8] which was used previously to evaluate geometries, spectra, and vibronic intensities of excimers in

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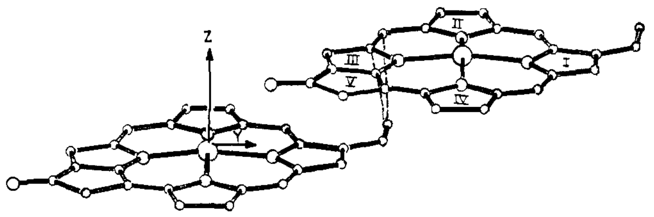

Figure 1. The dimer molecule included in the calculations. The coordinate system is attached to the center of the reference monomer where the Z axis is perpendicular to the plane of this monomer. The notation Z and Y in the paper designates the coordinates of the center of the second monomer and since the monomers are parallel Z is also the distance between their planes. The two bound water molecules are not drawn in the figure. The strongest p_σ interactions are indicated by --- (see ref 6). These interactions are found to give the largest contribution to the calculated red shift.

Table I. Calculated Shifts[a] of the Long-Wavelength Absorption of a Chlorophyll Dimer in a Nonpolar Solvent[b] and of a Crystal of Chlorophyll Derivative[c]

	contribution			Z, Å			
	Ex	overlap	solvent[d]	3.2	3.5	4.0	5.0
dimer	+	−	−	−384	−335	−273	−156
	+	−	+	−384	−335	−273	−156
	+	+	−	−760	−480	−355	−163
	+	+	+	−1110	−690 (−750)	−376	−174
crystal	+	+	+		−1240 (−1430)		

[a] In reciprocal centimeters. [b] The observed shift for P700 and related in vitro prepared dimers is given in brackets. Ex, overlap, and solvent indicate calculations including exciton interaction, p_σ overlap, and solvent effect, respectively. + and − indicate, respectively, including and not including a given contribution. For $Z = 3.5$ Å the two lowest calculated transitions are at 15 010 and 16 078 cm^{-1} and the corresponding oscillator strengths are 0.42 and 0.02, respectively. [c] The crystal geometry is evaluated by taking the same monomer geometry used for the dimer calculations and constructing the crystal lattice using the observed unit cell parameters and crystal symmetry.[6] The crystal shift is evaluated by constructing exciton and charge-transfer crystal wave functions of the type used in ref 19 and using our SCF Hamiltonian. The solvent shift term in the energy of the charge-transfer states is estimated by the method of ref 12. The observed crystal shift is given in brackets. [d] The calculated solvent shift is relative to the monomer in a nonpolar solvent.

molecular crystals.[9-10] The QCFF/PI approach treats the π electrons by a SCF-MO-CI semiempirical procedure and represents the effect of the σ electrons by empirical potential functions. The parameters used in the present work are those used in studies of structure, absorption spectra and resonance Raman spectra of metalloporphyrins.[11] The charges of the magnesium atom and the water molecule and the residual σ charges of the macrocycle are introduced as fixed charges in the π-electron-core Hamiltonian by the method of ref 12. The dimer molecule is treated as a supermolecule including the p_σ-type resonance integrals between the $2p_z$ orbitals on the different monomers and the hard core interaction between the σ electrons.[10] The effect of the solvent on the absorption spectrum is determined by our microscopic dielectric approach.[12,13] The geometry (Figure 1) is obtained by calculation of the equilibrium coordinates of the isolated monomers, placement of two monomers in the stacked orientation as observed in crystalline ethyl chlorophyllide *a*,[6] and adjustment of the out of plane displacements of the magnesium atom and the atoms of the ring I vinyl group to reproduce their observed positions.[6]

The dependence of the calculated shift of the long-wavelength absorption of the dimer on the distance between the monomer planes is given in Table I and Figure 2. As seen from Table I the calculation for $Z = 3.5$ Å accounts for the observed red shift in P700. Calculations based on the same parameters reproduce most of the observed red shift in a crystal of a chlorophyll derivative (see also Table I).

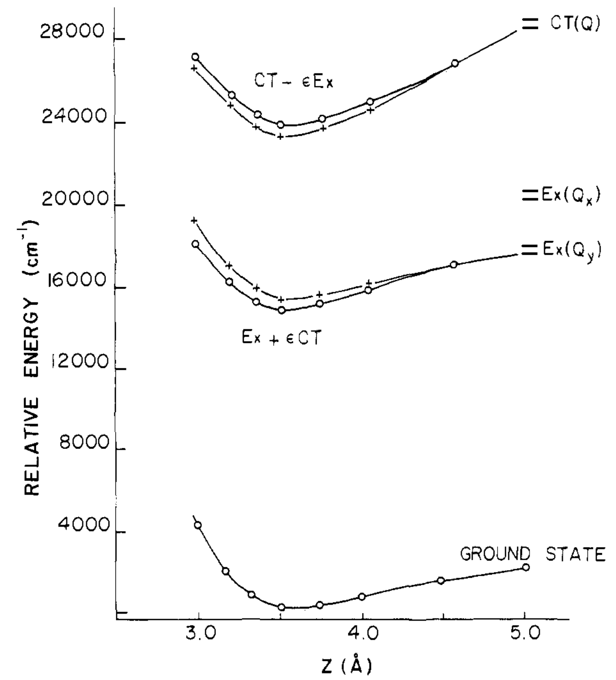

Figure 2. The dependence of the lowest lying exiton (Ex) and charge transfer (CT) states on the distance between the monomer planes (Z) for $Y = 8$ Å. The figure represents these states without and with overlap by (- + -) and (-O-) lines, respectively. Energies of other low lying states are given for $Z = 5$ Å, where Ex(Q_x) and Ex(Q_y) designate exciton states originating from the indicated monomer states and CT(Q) designates a CT state between the monomers' Q states.

It is very instructive to examine in Table I the different contributions to the calculated red shift. The exciton interaction contributes, at $Z = 3.5$ Å, less than half of the observed dimer shift. The second half is due to p_σ overlap between the monomers (especially to the interactions indicated in Figure 1). The p_σ overlap leads to the following two contributions. (1) A direct contribution is due to the splitting of the degenerate molecular orbitals (MO) of the dimer, where the energies of the antibonding MO increase and the energies of the bonding MO decrease (see Figure 2 of ref 14). As a result, the transition from the high antibonding occupied MO to the lowest unoccupied MO is red shifted. When the isolated molecule, rather than the supermolecule representation, is used, then the red shift is obtained by the interaction between the charge transfer and exciton states. Such an effect is well known in crystals of stacked aromatic molecules, where previous studies[9,15] indicated that *overlap must be introduced explicitly to account for the observed spectral shifts and for the stability of excimers.* Note that the p_σ bonding interaction between the monomers is obtained by ~30° twisting of the vinyl group.[6] Such a deformation, which decreases the conjugation in the isolated monomer, indicates that the dimer stacking interaction is achieved at the expense of increasing the energy of the isolated monomers. (2) The p_σ overlap leads indirectly to an additional red shift by inducing a significant solvent effect. That is, the mixing of the supermolecule dimer MO by the overlap interaction gives significant charge-transfer character to the lowest excimer state and this partially polar state is stabilized by the solvent. This effect is simpler to understand in the isolated representation where the energy shift of the exciton (Ex) state due to overlap interaction (β) with the charge-transfer (CT) state is given by

$$\Delta E_{\text{overlap}} = -\beta^2/(E_{\text{CT}} - E_{\text{Ex}}) \qquad (1)$$

Since the energy of the polar CT state is reduced by the solvent, we obtain significant increase in $\Delta E_{\text{overlap}}$ due to solvent effect. Obviously, when the p_σ overlap between the monomers is small ($Z > 4$ Å) this contribution to the red shift disappears. It is important not to confuse this overlap effect with the previously proposed solvent shift[2,3] (which is attributed to change of the *monomer*, rather than the dimer, dipole moment upon excitation). In fact, as seen from Table I, the solvent shift is zero in the present calculation in the absence of overlap interaction.[16]

In contrast to previous suggestions[2,3] the hydrogen bonds between the water and the V ring keto carbonyls are found to contribute a negligible red shift (6 cm^{-1}).

Recently, it was found that dimeric chlorophyll derivatives in which the macrocycles are bound by two covalent links do not display any appreciable red shifts.[17] However, in these dimers the monomers are held face to face ($Y \sim 0$, according to the notation of Figure 1). In such a system the allowed electronic transition is expected to be blue shifted rather than red shifted.

The observations that anhydrous dimers do not display large red shifts[2] was used as evidence that the water molecules in the (Chl $a{\cdot}H_2O)_2$ are responsible for a significant part of the observed red shift. We feel that the main contribution of the water is indirect, holding the dimer in a configuration with large excimer shift.

In summary, the present calculations demonstrate that the observed red shift in P700 can be reproduced by a dimer in a nonpolar solvent if the interaction between the monomer units is similar to that in the crystalline stack. In such a case, the red shift is primarily due to p_σ overlap and exciton interactions. We believe that the calculations are reliable since they also reproduce the observed red shift in a well-defined crystal of chlorophyll derivative.

Of course, our calculations do not exclude the possibility that in P700 or other reaction centers the monomers interact weakly and that the observed red shift is due to other factors (e.g., interaction with charged groups similar to that proposed for the red shift of retinal in rhodopsin[13]). However, it is clear now that, if the red shift of P700 is due to the same type of excimer interaction as that in the crystalline stack, then the distance between the monomers must be <4 Å; otherwise the overlap would be too small to account for the observed red shift. It is intriguing to note that excimer interactions are associated with extremely large Franck-Condon factors (see Table 2 of ref 9). A large Franck-Condon factor for the transition between the positively charged dimer and the neutral dimer might explain why the electron transfer in the photosynthetic process is so efficient.[18]

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Picosecond Studies of Excited-State Protonation and Deprotonation Kinetics. The Laser pH Jump†

Sir:

Among the most important and general chemical concepts are the acid–base properties of molecules. The Lewis definition of acids and bases, that an acid is an electron pair accepting species while a base is electron pair donating,[1] serves to demonstrate the link between the acid–base properties of a molecule and its electronic structure. Thus a change in electronic structure, e.g., by electronic excitation, should produce a concomitant change in acid–base properties. Such an effect was first observed by Weber,[2] who found a spectral shift in the fluorescence spectrum of 1-naphthylamine-4-sulfonate which occurred at a much different pH than the spectral shift in the absorption spectrum. Subsequent development by Förster[3] and Weller[4] has produced an active field of chemical research.[5] Such studies typically combine spectroscopic data on absorption and/or fluorescence with a Förster cycle[6] calculation to predict excited-state pK_a values. Because of the exceedingly rapid times scales involved, few direct determinations of the kinetics of excited-state proton-transfer reactions in solution have been made.[7,8]

By virtue of its extremely high time resolution, picosecond laser spectroscopy provides a general technique[9,10] for the study of excited-state proton-transfer reactions. We report here direct measurements of the kinetics of excited-state deprotonation and protonation for 2-naphthol-6-sulfonate and its anion in aqueous solution. In addition, a general method which utilizes the difference in ground- and excited-state pK_a values to provide a means for rapid initiation of acid- or base-catalyzed reactions, the laser pH jump, is described.

The basic experimental apparatus used in these experiments has been described in detail elsewhere[11] and is shown schematically in Figure 1. Briefly, a single 30-ps, 1060-nm pulse is extracted from the pulse train of a mode-locked Nd:YAG laser. A second harmonic, 532-nm pulse is generated in a KDP crystal and is in turn frequency doubled in ADP to produce the fourth harmonic at 266 nm. The 532- and 1060-nm pulses are removed by spectral filters. The 266-nm excitation pulse is directed onto the sample which is contained in an optical quality quartz cuvette of 2-mm path length. Fluorescence from the sample is imaged through the slit of a streak camera (Electrophotonics ICC-512, S-20 response) having a demonstrated resolution of 3 ps. The width of the 266-nm pulse was

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