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# Photophysics of the Variable Quantum Yield of Asymmetric Bilirubin

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*Abstract*

Bilirubin (BR), responsible for neonatal jaundice, is a 'Siamese twin' type of molecule containing two pyrromethenone chromophores conjoined by a saturated carbon $CH_2$ group. Because neonatal jaundice is cured by phototheraphy, bilirubin has been extensively studied by laser means. When the chromophores in each half of the molecule are identical, we have symmetrical BR (SBR); when they are not, we have antisymmetric BR (ASBR). The quantum yield of the photoproducts from SBR is not wavelength-dependent, while that from ASBR is, in organic solvents. Because of the proximity of the two chromophores, both the ASBR and SBR systems are subject to Davidov (dynamic electric dipole) splitting of the chromophore excited states. A quantum mechanical calculation shows that when the two (asymmetric) chromophore states are *not* degenerate, the higher Davidov state is preferentially occupied by the chromophore with the 'original' higher energy, and the lower Davidov state by the chromophore of 'original' lower energy. This is just what is required for the quantum yield to vary with wavelength. If the variation of the quantum yield of asymmetric bilirubin in the presence of human serum albumin is approximated by a square-wave (narrow line approximation), a quantum mechanical calculation of the ratio of the short wavelength photoproduct yield with the long wavelength one is in agreement with accepted values for the 'original' energy difference of the chromophores, and the Davidov splitting parameter.

## 1. Introduction

Bilirubins (BR) are tetrapyrroles with a primary structure shown in Fig. 1 (Fischer and Orth 1934–37). Bilirubins III$\alpha$ and XIII$\alpha$, denoted here by bil:$jj$ and bil:$ii$ respectively, are symmetric molecules with identical dipyrrinone groups. The vinyl groups in BR-III$\alpha$ are in the *exo* position, while they occupy the *endo* position in BR-XIII$\alpha$. Bilirubin IX$\alpha$ (bil:$ij$) consists of half of a BR-III$\alpha$ molecule bonded by a saturated $CH_2$ to half of the BR-XIII$\alpha$ molecule. Bilirubin IX$\alpha$ is then asymmetric.

Bilirubin IX$\alpha$ is the end product of the catabolic process of heme, from dead erythrocytes (Falk 1989). Its presence in large concentration in newborn infants is responsible for hyperbilirubinemia, i.e. neonatal jaundice. Bilirubins are photolabile and the study of their photophysics, photochemistry and metabolism has aroused strong interest since the discovery (Cremer *et al.* 1958) that exposure of icteric (jaundiced) infants to visible light increases the excretion of BR-IX$\alpha$ from the organism.

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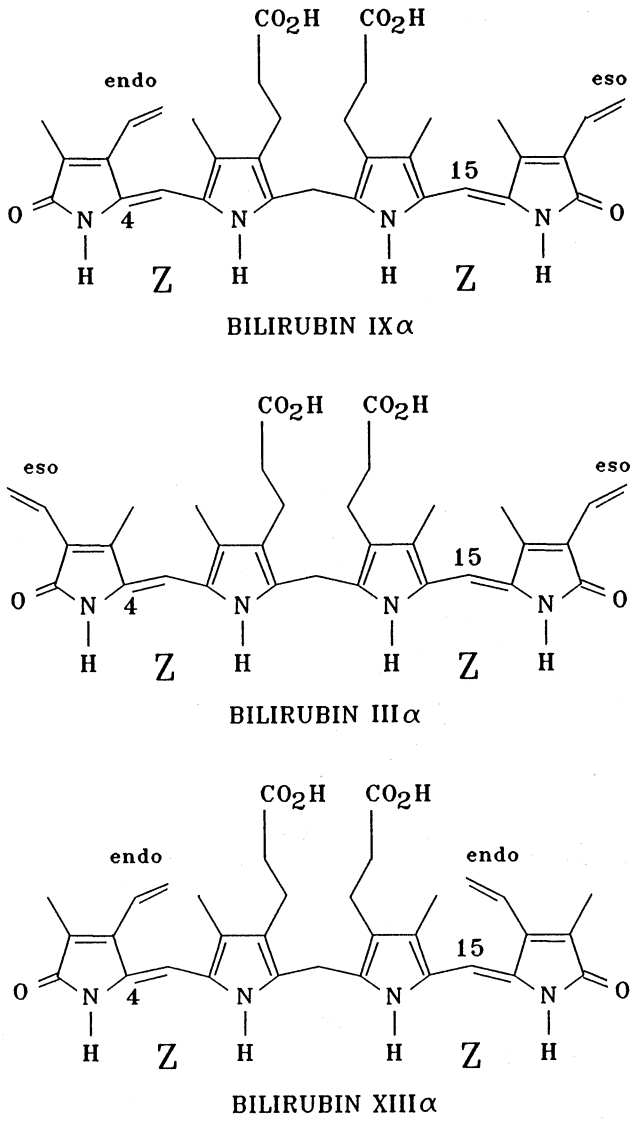


**Fig. 1.** Schematic diagrams of symmetric (IIIα and XIIIα) and asymmetric (IXα) bilirubins.

The discovery that bilirubins exhibit bisignate Cotton effects in circular dichroism spectroscopy (Blauer and Wagniere 1975) demonstrated that bilirubins are best described as pairs of interacting dipyrrinones (Lamola 1985). The interaction between the dipyrrinone chromophoric groups is assumed to be very weak in the unexcited molecule (Harada and Nakanishi 1983; Lightner *et al.* 1987), so light absorption is regulated by the cross sections of the two independent dipyrrinones. Excitation through one or the other dipyrrinone sets up a dipolar interaction between the two transition dipole moments of the chromophores, which is responsible for the development of the rotary strength (Schellman 1966).

More recently, several authors reported the dependence of the quantum yields $\phi$ for configurational (Pratesi *et al.* 1985; Agati *et al.* 1992) and structural (Greenberg *et al.* 1987; McDonagh *et al.* 1989) photoisomerisation of BR-IX$\alpha$ on the excitation energy. This wavelength dependence of quantum yields is not a frequent event in molecular photophysics, and has been considered to reflect unusual ground-state or excited-state properties of the molecule (Dauber *et al.* 1988).

The wavelength dependence of $\phi$ has been interpreted in terms of the exciton coupling mechanism between the two halves of the excited molecule (Lamola 1985; McDonagh and Lightner 1985*a*, 1985*b*). According to some qualitative descriptions (Lamola 1985; Greenberg *et al.* 1987; McDonagh and Lightner 1985; Agati and Fusi 1990), the interaction between the two dipyrrinone halves is sufficient to keep the excitation hopping back and forth between the two dipyrrinones until it is trapped in one or the other half. In the asymmetric BR-IX$\alpha$, energy dissipation in the *exo-* (*endo-*) vinyl half of the molecule through configurational change leads to the EZ (ZE) isomer. In aqueous complex with human serum albumin (HSA), the endo-vinyl side *appears* to be held much tighter in the albumin binding site than that with the exo-vinyl side, and only the ZE-BR-IX$\alpha$ isomer is formed.

We quote Lamola (1985): 'From the reported absorption spectra of $Z,Z$-bilirubin-III$\alpha$ and XIII$\alpha$ in various solvents, it is suggested that the long-wavelength transition in the dipyrromethone half with the *exo* vinyl group (through conjugation) lies 300 $\mathrm{cm}^{-1}$ lower than that of the half with the *endo* vinyl group (cross conjugation). Of course, interactions with the microenvironment, say in a protein, could easily reverse this state ordering.'

Until now, only pictorial descriptions and semiquantitative estimates of the exciton coupling mechanism for the wavelength dependence of the photoisomerisation quantum yields have been provided. In this article, a quantum mechanical analysis of the photophysics of excited BR-IX$\alpha$ is presented. An estimate of the variation of the configurational quantum yield of BR-IX$\alpha$ with excitation energy is also presented and compared with experimental results.

## 2. Quantum Mechanical Model of BR-IX$\alpha$

The asymmetric bilirubin IX$\alpha$, denoted here by bil:$ij$, consists of two similar, but not identical dipyrrinone chromophores, denoted here by dp:$i$ and dp:$j$. In dp:$i$ (dp:$j$) the vinyl group is in the endo (exo) position. According to molecular exciton theory applied to binary systems, the quantum state of the total system bil:$ij$ in the singlet excited state consists of an excited state doublet $|ij\rangle_{\alpha,\beta}$ represented by the linear combination:

$$|ij\rangle = C_i^{\alpha,\beta}|ia,\, j0\rangle + C_j^{\alpha,\beta}|0,\, jb\rangle \tag{1}$$

of the states $|ia,\, j0\rangle = |ia\rangle\,|j0\rangle$, in which the chromophore $i$ *(j)* is in the excited state $a$, energy $E_a$ (ground state 0), and $|i0,\, jb\rangle = |i0\rangle\,|jb\rangle$, in which the chromophore $i$ $(j)$ is in the ground state 0 (excited state $b$, energy $E_b > E_a$) (ground state energies are assumed equal to zero). The coefficients $(C_i^{\alpha,\beta})^2$ and $(C_j^{\alpha,\beta})^2$ represent the probabilities of localisation of energy in the chromophores

$i$ and $j$, and for levels $\alpha$ and $\beta$, respectively. The coefficients and perturbed energy levels are calculated in the Appendix. We have

$$(C_i^\alpha)^2 = (C_j^\beta)^2 = \frac{\sqrt{1+\Delta^2}+\Delta}{2\sqrt{1+\Delta^2}}, \tag{2}$$

$$E^{\alpha,\beta} = \tfrac{1}{2}(E_a + E_b) \mp \tfrac{1}{2}\sqrt{(E_a - E_b)^2 + 4V_{ij}^2}, \tag{3}$$

where $\Delta \equiv (E_b - E_a)/2\Delta_{ij}$, and $V_{ij}$ denotes the Davidov interaction matrix element.

In the case of a binary system composed of two identical chromophores $i$ and $j$, such as BR-III$\alpha$ and BR-XIII$\alpha$, the chromophore energy levels are degenerate in the composite molecule ($E_a = E_b$, $\Delta = 0$), and equations (2) and (3) give $(C_i^\alpha)^2 = (C_j^\alpha)^2 = (C_i^\beta)^2 = (C_j^\beta)^2 = \frac{1}{2}$; $E^\beta - E^\alpha = 2V_{ij}$ (Davidov splitting). Therefore, the exciton (Davidov) splitting gives an equal occupation of each $|i\rangle$, $|j\rangle$ state for each exciton state $|ij\rangle_{\alpha,\beta}$. For these symmetric molecules it is clear that the quantum yield of each will not vary with excitation wavelength, though the photoproducts will be different (i.e. ZE-BR-III$\alpha$ and EZ-BR-III$\alpha$ for BR-III$\alpha$, etc.).

In the asymmetric BR-IX$\alpha$ molecule the chromophore energy levels associated with the states $|i\rangle$, $|j\rangle$, $E_a$ and $E_b$, respectively, are no longer degenerate with, say, $E_b > E_a$. Thus, a 'pre-Davidov' splitting $E_b - E_a$ makes the occupation of the $|i\rangle$, $|j\rangle$ states in the exciton states unequal. Hence the different chromophores $i$ and $j$ are not equally excited across the absorption line of bil: $ij$, and the quantum yield will vary. The probability of localisation of energy in chromophore $i$ for energy level $E^\alpha$, $(C_i^\alpha)^2$, increases monotonically as a function of $\Delta$ from the value 0·5 for $\Delta = 0$ (degenerate system) to 1 for $\Delta \gg 1$. Since, from equation (2), $(C_i^\alpha)^2 = (C_j^\beta)^2$, the $|i\rangle$ state will be more favoured in the lower exciton state, and the $|j\rangle$ state will be equally more favoured in the upper exciton state.

For example, if $\Delta = 2$, then we have $(C_i^\alpha)^2 = 0{\cdot}85$. Thus, a 'pre-Davidov' splitting equal to the Davidov splitting gives an 85% occupation of whichever chromophore is excited. This is just what is needed for the higher energy chromophore to be preferentially excited at higher energy, and the lower one at lower energy.

If the rate of photochemical transformation is slow compared to the 'hopping' between the two exciton states, an average (non-varying) quantum yield over all excitation wavelengths might still be expected, rather than an excitation wavelength dependence. However, this is simply not the case with bilirubin; the relaxation from either chromophore excited state to the ground state is extremely rapid, as is the photoproduct formation (Lightner and McDonagh 1984).

## 3. Comparison with the Experiments

According to the above theory, the *observed* quantum yield for the formation of the ZE-BR-IX$\alpha$ isomer, $\phi_{\mathrm{ZZ}\to\mathrm{EZ}}$, due to photochemistry in the chromophore $j$ with the *endo* configuration, is given by the quantum yield $\phi^*{}_{\mathrm{ZZ}\to\mathrm{ZE}}$ in the absence of exciton coupling weighted by the probability of occupation of the state $|j\rangle$, i.e. $(C_j^\beta)^2$ at high energy and $(C_j^\alpha)^2$ at low energy. Similarly, the quantum yield $\phi^*_{\mathrm{ZZ}\to\mathrm{ZE}}$ for photochemistry occurring in the exo chromophore $i$ has a weight

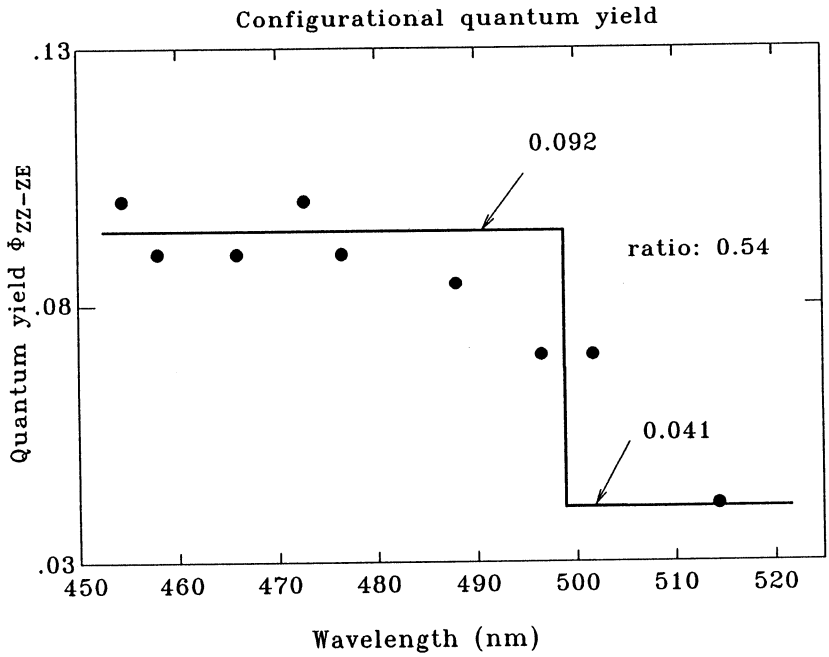


**Fig. 2.** Approximation of the variation of the quantum yield of asymmetric bilirubin (IX$\alpha$) by a step function.

factor $(C_i^{\beta})^2$ and $(C_i^{\alpha})^2$ at high and low excitation respectively. Thus, the ratio $\phi_{EZ}/\phi_{ZE}$ between the observed quantum yields contains the factor $\chi \equiv (C_j^{\beta}/C_i^{\beta})^2$ for relaxation from the upper exciton state, and the factor $(C_j^{\beta}/C_i^{\beta})^2 = 1/\chi$ for relaxation from the lower exciton state. For $\Delta = 2$, the factor is $\chi \sim 6$, and a reduction by a factor ~36 is expected for the ratio $\phi_{EZ}/\phi_{ZE}$ when the excitation energy is lowered.

To date, no measurements of the configurational quantum yields of BR-IX$\alpha$ versus wavelength are available in organic solvents, where both isomers are formed.

When bound to human serum albumen (HSA), BR-IX$\alpha$ isomerises only to the ZE form on light excitation. The quantum yield $\phi_{ZZ \rightarrow ZE}$ of BR-IX$\alpha$/HSA has been found to decrease with an increase of excitation wavelength from ~0·1 to ~0·05. The high energy chromophore $|j\rangle$ is then assumed to be the dipyrrinone with endo vinyl group (Lamola 1985). According to Lamola, the long-wave transition in the dipyrrinone half with the exo vinyl group lies 300 $cm^{-1}$ lower than that of the half with the endo vinyl group, and the dipolar interaction between the transition moments of the two halves, $V_{ij}$, is approximately 500 $cm^{-1}$ for a 'random' conformation of BR, but the situation can be reversed (see the quotation from Lamola in the Introduction). Thus, $\Delta \approx 0{\cdot}3$, and (from equation 2) $(C_i^{\beta})^2 \approx 0{\cdot}36$ $(\sim\frac{1}{3})$, $(C_i^{\alpha})^2 \approx 0{\cdot}64$ $(\sim\frac{2}{3})$, and $(C_i^{\beta}/C_i^{\alpha})^2 \approx \frac{1}{3}\times\frac{3}{2} \approx 0{\cdot}55$ $(\sim\frac{1}{2})$. The quantum yield $\phi_{ZZ \rightarrow ZE}$ is then expected to decrease by a factor of $\sim\frac{1}{2}$ when the excitation energy is decreased. This is just what is observed experimentally, with remarkably good agreement, as shown in Fig. 2 where a plot of $\phi_{ZZ \rightarrow ZE}$ versus wavelength is reported. After all, 'the square wave' approximation to the quantum yield assumes the 'narrow line' (delta function) approximation to the absorption lines.

## 4. Discussion

The narrow linewidth approximation is valid, since Lamola (1985) gave the *upper limit* to the linewidth at half height as 2500 $cm^{-1}$ with a centre wavelength of 450 nm. This gives a linewidth to centre frequency ratio of $\sim 0 \cdot 11 \times 10^3$. The product state approximation used means that, other than the Davidov interaction, there is very little interaction between the chromophores $i$ and $j$, which is usually taken to be the case. However, the above explanation for the variable quantum yield of asymmetric bilirubin, even though it gives only the high- and low-wavelength asymptotic values, must be a good part of the story. The more complicated model described in the Introduction, involving potential wells in the excited states, is much more difficult to calculate, and in fact in all accounts of this model no mechanism is given for the selection of one chromophore excited state over the other in the different wavelength regions.

The dependence of photoproduct quantum yield for the symmetric bilirubins bound to HSA has not yet been reported in the literature. While this might be expected not to vary because of the molecular symmetry, there is the possibility that bilirubin may be asymmetrically *bound* to HSA, thus breaking the symmetry and splitting the originally identical chromophore levels sufficiently for chromophore selection with wavelength to take place, again causing a dependence of photoproduct quantum yield with excitation wavelength.

A preliminary account of this work was presented at the ACOLS 93 conference at Melbourne University, Australia, in 1993.

*Note added in proof*

Recently, the variation of the quantum yield with wavelength has been studied in the following systems: (a) symmetric and asymmetric bilirubins in 0·1% ammonia/methanol solution; (b) SBRs in human serum albumin aqueous buffer solution. In (a), the quantum yield does not vary with wavelength for the SBRs, while for the ASBRs it does. In (b), the quantum yield varies with wavelength for all SBRs, showing they are asymmetrically bound to HSA. These results are being prepared for publication (G. Agati 1995; personal communication).

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## Appendix: Occupation of Exciton States by Chromophore States

It has already been shown by Harada and Nakanishi (1983) that if the chromophore energy states (no exciton coupling assumed) are non-degenerate, then the occupation of the exciton states by the chromophore states $|i\rangle$, $|j\rangle$ will be asymmetric. Let the energies of the upper states of the chromophore be $\sigma_a$ for pm: $i$ and $\sigma_b$ for pm: $j$, before the Davidov interaction with matrix element $V_{ij}$ is 'switched on'. Then we have in the notation of Harada and Nakanishi for the *total* (Davidov) states $\alpha, \beta$, with energies $E^\alpha < E^\beta$,

$$E^\alpha = \tfrac{1}{2}(\sigma_\alpha + \sigma_\beta) - \tfrac{1}{2}\{(\sigma_b - \sigma_a)^2 + 4V_{ij}^2\}^{\frac{1}{2}},$$

$$\psi^\alpha = C_i^\alpha \, \phi_{ia} \, \phi_{j0} + C_j^\alpha \, \phi_{i0} \, \phi_{jb},$$

$$E^\beta = \tfrac{1}{2}(\sigma_a + \sigma_b) + \tfrac{1}{2}\{(\sigma_b - \sigma_a)^2 + 4V_{ij}^2\}^{\frac{1}{2}},$$

$$\psi^\beta = C_i^\beta \, \phi_{ia} \, \phi_{j0} + C_j^\beta \, \phi_{i0} \, \phi_{jb},$$

and the ground state is $\psi_0 = \psi_{i0} \, \psi_{j0}$. The transition moments are

$$\langle 0|\mu|\alpha\rangle = C_i^\alpha \, \mu_{i0a} + C_j^\alpha \, \mu_{j0b}, \qquad \langle 0|\mu|\beta\rangle = C_i^\beta \, \mu_{i0a} + C_j^\beta \, \mu_{j0b},$$

$$\mu_{i0a} = \int \phi_{i0} \, \mu_i \, \phi_{ia} \, \mathrm{d}\tau_i, \qquad \mu_{j0b} = \int \phi_{j0} \, \mu_j \, \phi_{jb} \, \mathrm{d}\tau_j .$$

The coefficients $C_i^\alpha, C_j^\alpha, C_i^\beta, C_j^\beta$ are calculated from the matrix eigenvalue equation transposed, e.g. $(\sigma_a - E^\alpha) C_i^\alpha + V_{ij} \, C_j^\alpha = 0$, and the normalisation condition $(c_i^\alpha)^2 + (c_j^\alpha)^2 = 1$. Hence we have

$$C_i^\alpha = V_{ij} / \{V_{ij}^2 + (\sigma_a - E^\alpha)^2\}^{\frac{1}{2}},$$

$$C_j^\alpha = \{(\sigma_a - E^\alpha)^2\}^{\frac{1}{2}}.$$

Since we can set $C_i^\alpha = \cos\theta = c$ and $C_j^\alpha = \sin\theta = s$, then by orthogonality of eigenvectors we have $C_i^\beta = s$, $C_j^\beta = -c$, or equivalent. This equivalence gives

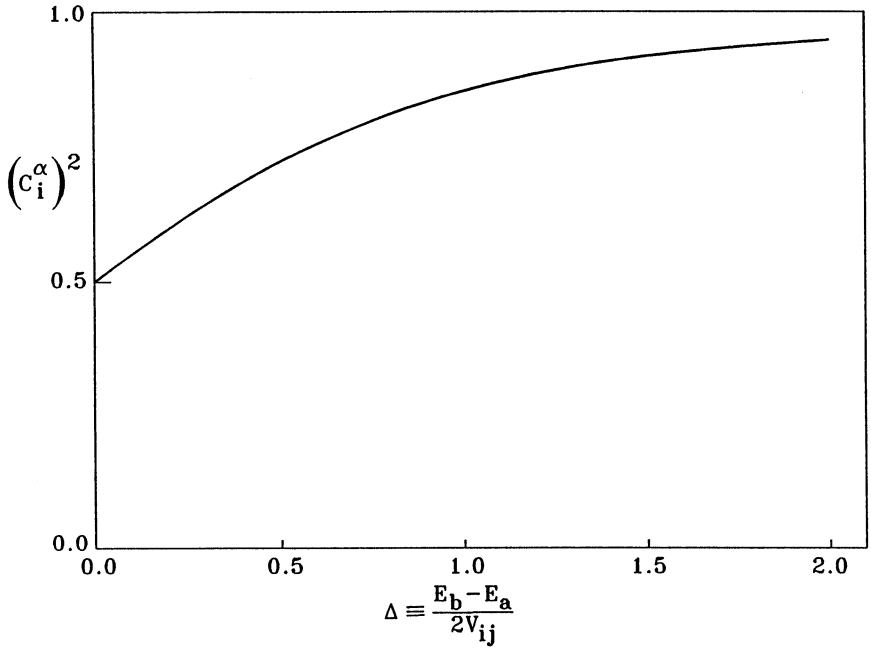


**Fig. 3.** The coefficient $(C_i^\alpha)^2$ plotted against the ratio $\Delta = (E_b - E_a)/V_{ij}$.

$C_i^\alpha C_j^\alpha = -C_i^\beta C_j^\beta = V_{ij}/t$, where $t = \{(\sigma_b - \sigma_a)^2 + 4V_{ij}^2\}^{\frac{1}{2}}$ is proved (at length) by Harada and Nakanishi (1983). We use again their notation:

$$\delta = \sigma_b - \sigma_a, \qquad t = \{(\sigma_b - \sigma_a)^2 + 4V_{ij}^2\}^{\frac{1}{2}},$$

whence $4V_{ij}^2 = t^2 - \delta^2$ and $\sigma_a - E^\alpha = (t-\delta)/2$, and therefore

$$\begin{aligned}\{V_{ij}^2 + (\sigma_a - E^\alpha)^2\}^{\frac{1}{2}} &= \left(\frac{t^2 - \delta^2}{4} + \frac{(t-\delta)^2}{4}\right)^{\frac{1}{2}} \\ &= \left(\frac{(t-\delta)}{4}(t + \delta + t - \delta\right)^{\frac{1}{2}} \\ &= \left(\frac{t(t-\delta)}{2}\right)^{\frac{1}{2}},\end{aligned}$$

$$C_i^\alpha = \left[\frac{t^2 - \delta^2}{4}\right] \Big/ \left(\frac{t(t-\delta)}{2}\right)^{\frac{1}{2}} = \left[\frac{t+\delta}{4}\right]^{\frac{1}{2}} \Big/ (t/2)^{\frac{1}{2}},$$

$$C_j^\alpha = -\left[\frac{t-\delta}{2}\right] \Big/ \left(\frac{t(t-\delta)}{2}\right)^{\frac{1}{2}} = -\left[\frac{t-\delta}{4}\right]^{\frac{1}{2}} \Big/ (t/2)^{\frac{1}{2}}.$$

This is not as useful as writing $\eta = 2V_{ij}$ and using the ratio $\delta/\eta = \Delta$. We then have $t = (\delta^2+\eta^2)^{\frac{1}{2}} = \eta(\Delta^2+1)^{\frac{1}{2}}$, $\delta = \eta\Delta$. This is a good basis for calculating $C_i^\alpha$, $C_j^\alpha$, etc.:

$$c = C_i^\alpha = \frac{\{\eta(\Delta^2+1)^{\frac{1}{2}} + \eta\Delta\}^{\frac{1}{2}}}{2[\eta(\Delta^2+1)^{\frac{1}{2}}/2]^{\frac{1}{2}}} = \frac{\{(\Delta^2+1)^{\frac{1}{2}} + \Delta\}^{\frac{1}{2}}}{\sqrt{2}(\Delta^2+1)^{\frac{1}{4}}},$$

$$s = C_j^\alpha = \frac{\{\eta(\Delta^2+1)^{\frac{1}{2}} - \eta\Delta\}^{\frac{1}{2}}}{2[\eta(\Delta^2+1)/2]^{\frac{1}{2}}} = \frac{\{(\Delta^2+1)^{\frac{1}{2}} - \Delta\}^{\frac{1}{2}}}{\sqrt{2}(\Delta^2+1)^{\frac{1}{4}}}.$$

Now $|c^2|$ is the probability of localisation of energy in chromophore $i$ for energy level $E^\alpha$, equal to the probability of localisation of energy in chromophore $j$ for energy level $E^\beta$. This is shown in Fig. 3 as a function of $\Delta$ (remember $|c^2| + |s^2| = 1$).

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