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PICOSECOND LASER SPECTROSCOPY AND OPTICALLY DETECTED MAGNETIC RESONANCE ON A MODEL PHOTOSYNTHETIC SYSTEM

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Abstract—Fluorescence-detected magnetic resonance of triplets in zero magnetic field (FDMR), fluorescence fading (FF) due to triplet-formation, both at 4.2 K, and prompt fluorescence decay kinetics (FDK) at room temperature have been measured for free pheophorbide-*a* (f-Pheo) and bound (b-Pheo) to a synthetic polypeptide (L-LYS-L-ALA-L-ALA)$_n$, dissolved in dimethylformamide (DMF). Fluorescence decay kinetics measurements of f-Pheo in DMF yielded 1–5 ns lifetimes, for b-Pheo in DMF a ~ 50 ps decay-component was found emitting at 730–750 nm. Zero-field splitting parameters |D| and |E| of the lowest triplet state T_1 were determined from FDMR spectra as (337 and 24) × 10^{-4} cm^{-1} for f-Pheo and (359 and 25) × 10^{-4} cm^{-1} for b-Pheo, both in DMF. Decay rate constants of the three spin levels of T_1 of b-Pheo (k_x = 1200 ± 50 s^{-1}, k_y = 440 ± 25 s^{-1}, k_z = 80 ± 5 s^{-1}) and relative steady-state populations (N_x = 28 ± 2%, N_y = 47 ± 2%, N_z = 26 ± 2%) determined from FF curves predict a fluorescence decrease at the D−E and D+E FDMR transitions, whereas experimentally a fluorescence increase is observed. The FDMR sign-inversion results from singlet-singlet energy transfer from b-Pheo monomers to their aggregates, followed by fast intersystem crossing to T_1. These results indicate that aggregates are formed by two or more b-Pheo molecules at different positions on the folded polypeptide chain. This situation resembles that in chlorophyll-proteins, containing low-lying traps, resulting from interaction of chromophores with other chromophores and with the protein environment.

INTRODUCTION

In recent years energy transfer in isolated photosynthetic systems has been widely studied using various spectroscopic techniques, including optically detected magnetic resonance (ODMR)† (Clarke, 1982), fluorescence fading (FF) (Van Dorp *et al.*, 1973) Avarmaa, 1977, 1979), and fluorescence decay kinetics (FDK) (Pellegrino and Alfano, 1982; Karukstis and Sauer, 1983; Searle *et al.*, 1983). A major, fast (~ 40 ps) fluorescence component has been found in the long wavelength emission of antenna chlorophyll-*a* (Chl-*a*) of Chl-*a* protein 1 from the plant photosystem I, and has been ascribed to singlet energy transfer to the reaction center (RC) (Searle *et al.*, 1983). The Chl-*a* triplet FDMR spectra of this protein exhibit a striking sign-inversion when compared to what is predicted from FF measurements on the same protein. This phenomenon can also be explained by energy transfer from the antenna to the RC. A similar interpretation has been presented for the sign-inversion in FDMR spectra of whole algal cells (G. H. van Brakel, The triplet state of chlorophyll-*a* in whole algal cells, Thesis, Agricultural University, Wageningen, 1982) and photosynthetic bacteria (Hoff *et al.*, 1981; J. Beck, ODMR on pigment-complex of photosynthetic bacteria, Thesis, University of Stuttgart, Stuttgart, 1983).

It is the purpose of this paper, to present a model, explaining results of fluorescence and magnetic resonance measurements on a synthetic (L-Lys-L-Ala-L-Ala)$_n$ polypeptide with covalently bound pheophorbide-*a*. Such a model may be applied to chlorophyll–protein complexes containing an active RC, which exhibit strikingly similar results, using the same techniques.

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†*Abbreviations*: A, aggregated; b-Pheo, pheophorbide-*a* bond to a synthetic polypeptide; Chl-*a*, chlorophyll-*a*; CW, continuous wave; C, concentration; DMF, dimethylformamide; FDK, fluorescence decay kinetics; FDMR, fluorescence detected magnetic resonance; FF, fluorescence fading; f-Pheo, free pheophorbide-*a*; M, monomeric; ODMR, optically detected magnetic resonance; Pheo, pheophorbide-*a*; RC, reaction center; ZFS, zero field splitting.

MATERIALS AND METHODS

Pheophorbide-*a* (Pheo) was made from Chl-*a* (Vavrinec and Skorkovska, 1973) using selective hydrolysis (Wasielewski and Svec, 1980). The synthesis of the model peptide was carried out by a polymerization of the tripeptide L-Lys-L-Ala-L-Ala (Blaha *et al.*, 1976). Pheo was covalently bound to the synthetic polypeptide by alink between the ε-amino group of the lysine side-chain in the sequential polytripeptide and the C 17 carboxyl group of Pheo (Fig. 1). The bound complex (b-Pheo) could be dissolved in

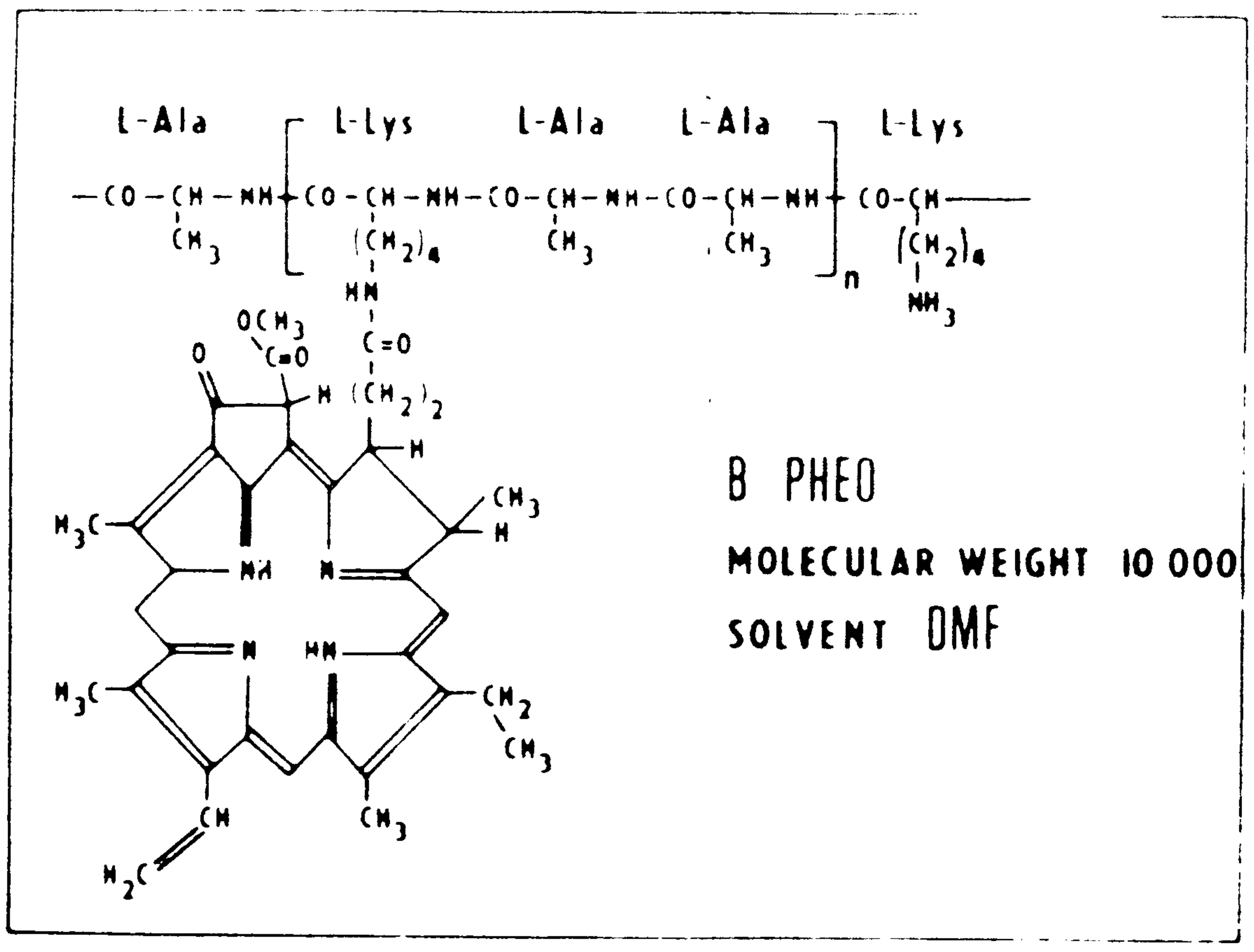

Figure 1. Structural formula of the photosynthetic model of pheophorbide-*a* covalently bound to polypeptide.

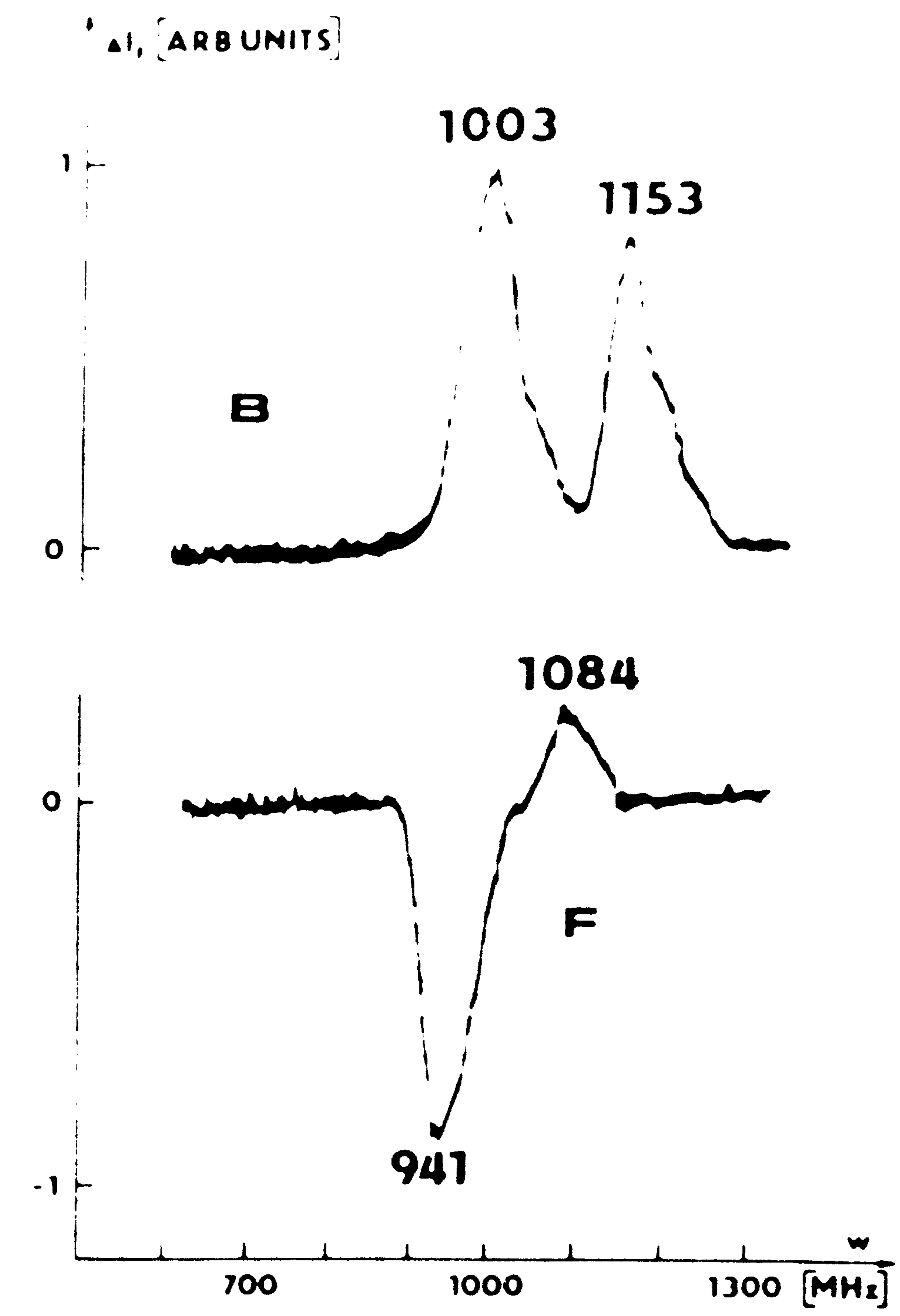

Figure 3. 4.2 K FDMR spectra of 2×10^{-6} *M* solutions of f- and b-Pheo in DMF excited at 501.7 nm (Ar⁺), 100 mW; detection at 667.5 ± 2.5 nm (f-Pheo), 669 ± 2.5 nm (b-Pheo).

DMF (Fluka, UV) up to 2×10^{-6} *M* by means of ultrasonic treatment. Free pheophorbide-*a* (f-Pheo) was studied in 10^{-5}–10^{-7} *M* DMF and *n*-octane (Baker, Anal. Grade) solutions.

Absorption- and fluorescence-spectra were measured on a Uvikon 810 spectrometer (Kontron) and a MFF-2a fluorimeter. For low temperature measurements, we used an Oxford Instruments CF204 helium flow cryostat. Absorption- and fluorescence-spectra of f-Pheo and b-Pheo in DMF at room temperature are shown in Fig. 2. FDMR and FF experiments were performed as described by Schaatsma (in Clarke, 1982) and Avarmaa (1977). For optical excitation, we used a 900 W Xenon arc (Osram), and a combination of a saturated $CuSO_4$ solution, and Shott BG 12 and GG 395 filters, resulting in an excitation band of 370–465 nm. In some experiments we used the 501.7 nm line of a CW Ar^+ laser (Coherent Radiation CR-4) as a light-source. The fluorescence of the sample was detected using a Shott RG 610 filter and a 0.25 Spex Minimate, equipped with a cooled (−30°C) RCA C31034A or Varian VPM 1520 photomultiplier. Signals were recorded on a PAR C-4203 averager, and stored in a MINC-11 computer. In FDMR measurements we applied saturating microwavepower from a HP 8620C/86220A 10-1300 MHz sweep generator, equipped with a Mini-circuits Lab. (27 db) wave amplifier, to the sample, placed in a helix, submersed in liquid He. Resonance frequencies were determined using a Systron-Donner 1017 frequency counter, having a typical error of ± 10 MHz. In FF measurements, chopped laser excitation at different intensities was used (0–200 mW, ~20 Hz). The FF curves were analysed by a least squares fit to a sum of one to four exponentials

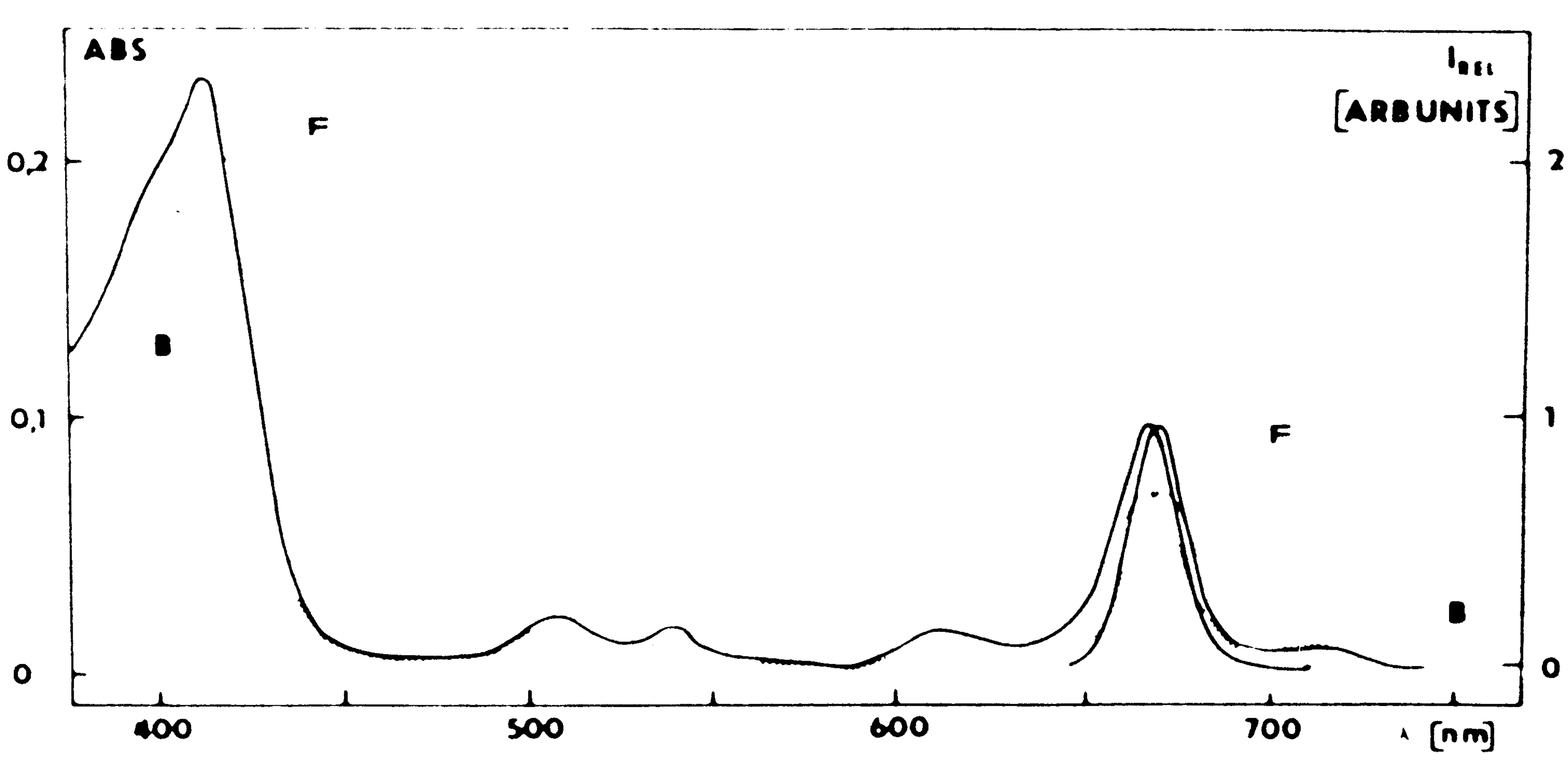

Figure 2. Absorption- and fluorescence spectra (excited at 610 nm), of f- and b-Pheo in DMF; T = 300 K, C = 2×10^{-6} *M*

(Provencher, 1976). Lifetimes of prompt fluorescence were measured by single photon counting at different emission wavelengths using an 18 W Ar ion laser (Coherent Radiation CR 18 UV) with acousto-optic modelocking and a synchronously pumped Rhodamin 6G dye laser (CR 590 producing ~5 ps excitation pulses at a repetition rate of 297.5 kHz employing an electro-optic modulator. Fluorescence photons from the sample solution in a 10 mm quartz cuvette passed through two 0.25 m Jarrell-Ash monochromators (resolution ~10 nm) and were detected using a Philips PM 2254 B photomultiplier, a constant fraction discriminator (Camberra 1428 A) and Nuclear Data 66 Multichannel Analyzer. The instrument has a typical time-resolution of ~50 ps, using a standard deconvolution methd and a least-squares fit on a DEC-10 computer to a sum of up to three exponentials (Visser and van Hoek, 1979, 1981). For a more detailed description of the apparatus see van Hoek and Visser (1981) and Searle *et al.* (1983).

RESULTS

The 4.2 K FDMR spectra of f-Pheo in -octane + 0.5% ethanol (10 [illegible] *M*) and DMF (2 × 10 [illegible] *M*) consist of a strong D − E peak with negative amplitude (i.e. corresponding to a decrease of fluorescence) and a weak positive D + E peak (Fig. 3). The transition at 2E has a low, negative amplitude and is often not observed, most likely due to the low level of microwave radiation at this frequency (Benthem, 1984). The spectrum of b-Pheo in DMF at the same concentration is very different, showing two strong positive peaks. Zero-field splitting (ZFS) parameters |D| and |E| derived from the FDMR transition frequencies observed at D − E and D + E have been collected in Table 1 for free/bound Pheo and pheophytin-a for comparison. Attempts to detect FDMR in the 730 nm aggregate fluorescence band were unsuccessful.

The relative steady-state populations $N_{i,j,k}$ of the triplet spin-levels, their decay-constants $k_{i,j,k}$, and their relative population rates $P_{i,j,k}$, were determined from FF measurements. In FF experiments, the time-dependent response of the fluorescence intensity to an excitation stepfunction, due to ingrowth of the triplet-population is given by (Avarmaa, 1977, 1979)

$$I_f(t) = I_f(\infty) + [I_f(O) - I_f(\infty)] \frac{\sum_{m=i,j,k} C_m \exp(-k_m t)}{\sum_{m=i,j,k} C_m} \quad (1)$$

where i,j,k label the zero-field triplet spin-levels; furthermore,

$$N_m = \frac{P_m}{k_m} = \frac{C_m}{\sum_m C_m} \quad (2)$$

At sufficiently low excitation rate, k_m depends linearly on the fraction of molecules excited into the triplet state, given by $1 - I_f(\infty)/I_f(O)$. By extrapolating to zero excitation intensity, corresponding to zero fractional triplet population, the true molecular kinetic constants k_m and P_m ($P_m = N_m k_m$) were obtained (Avarmaa, 1979; Avarmaa and Schaafsma, 1980), as summarized in Table 2. This table also contains the same kinetic constants, measured at an excitation intensity that was used during FDMR experiments, and the predicted and observed signs of the D − E and D + E FDMR transitions of f-Pheo and b-Pheo using these kinetic constants.

The fractional steady-state triplet population using 200 mW laser-excitation intensity of b-Pheo is found to be twice as large as for f-Pheo under the same conditions.

Fluorescence decay kinetics of both f- and b-Pheo in DMF were measured at various emission-wavelengths. Excitation was into a vibronic satellite of the red absorption band at 610 nm. The lifetimes τ_i and relative amplitudes α_i found after fitting the data to $I_f(t) = \sum_i \alpha_i \exp(-t/\tau_i)$ are collected in Table 3.

DISCUSSION

Although the f-Pheo ZFS values are quite sensitive to solvent-polarity (c.f. data in *n*-octane and DMF), the ZFS values of b-Pheo in the polar DMF solvent are close to those of f-Pheo in strongly apolar *n*-octane solution (Table 1). This indicates, that the environment of the Pheo-triplets in b-Pheo is strongly apolar. Evidently, the rather apolar pheophorbide-moieties are buried in the hydrophobic core of the polypeptide, which exposes its charged lysine side-groups to the polar DMF solvent (see Fig. 4). This model suggests that the folding of the peptide may cause some chromatophores to come within close range, and is supported by a number of experimental results:

Decay rate-constants k_m (m = i,j,k) from FF experiments (Table 2) are similar for f- and b-Pheo, but the relative steady-state populations N_m of T_1 are very different, both at very low and non-zero steady-state fractional triplet population. This means, in fact, that the relative populating rates of the three T_1 spin levels are different for both compounds (c.f. Eq. 3), as is also reflected by the ~ two-fold increase of the triplet yield of b-Pheo as compared to that of f-Pheo (Table 2). These results can be understood by assuming that for the species observed by FF, the energy-gap between T_1 and the groundstate (S_0) is approximately equal for f-Pheo and b-Pheo, leaving the T_1 decay rate-constants almost unchanged. On the other hand, the S_1-T_1 gap should be smaller for b-Pheo, than for f-Pheo, in order to explain the difference in populating rates. Necessarily, this means that S_1 is at lower energy in b-Pheo than in f-Pheo. The drop of the lowest excited singlet state is ascribed to excitonic interaction (Kasha *et al.*, 1965; Kasha, 1976) between two or more Pheo molecules at close range, labeled A (aggregated) in Fig. 4. The majority of

Table 1. ZFS parameters of free/bond pheophorbide-*a* and pheophytin-*a*; T = 4.2 K

Species	Solvent	Conc. (*M*)	$\lvert D \rvert (\times 10^{-4} cm^{-1})$	$\lvert E \rvert (\times 10^{-4} cm^{-1})$
f-Pheo	*n*-octane/ 0.5% ethanol	10^{-5}	359 ± 2	27 ± 1
f-Pheo	DMF	2×10^{-6}	337 ± 2	24 ± 1
b-Pheo	DMF	2×10^{-6}	359 ± 2	25 ± 1
Pheophytin-*a*	*n*-octane/ 0.5% ethanol	10^{-5}	350 ± 2*	20 ± 1*

*Data from van der Bent and Schaafsma (1975b).

Table 2. Kinetic data and predicted/observed signs of FDMR transitions of the lowest triplet state of f- and b-Pheo. T = 4.2 K

Compound*	ΔT†/N (%)	k_x	k_y s^{-1}	k_z	N_x	N_y (%)	N_z	Sign (calc.)‡ D−E	Sign (calc.)‡ D+E	Sign (exp.)§ D−E	Sign (exp.)§ D+E
f-Pheo	→ 0	1367 ± 135	409 ± 31	55 ± 9	22.5	19.9	57.6				
	4–7‖	1550–1850	550–650	130–170	25–27	39–48	36–25	−	+	−	+
b-Pheo	→ 0	1199 ± 47	444 ± 24	82 ± 6	27.6	46.8	25.6				
	8–13‖	1400–1500	520–570	110–130	33–35	43–41	24	−	−	+	+

*Solvent for both compounds: DMF; †Fraction of total number (N) of molecules in the triplet state, determined from $[I_F(0) - I_F(\infty)]/[I_F(0)]$ of the FF curve; ‡Calculated from Eq. (3); §Detected at 670–680 nm, see Fig. 3; ‖Range of values for 100–200 mW 501.7 nm Ar^+ excitation; fractional triplet-population of b-Pheo is always ~ twice that of f-Pheo, measured under identical conditions.

Table 3. Fluorescence decay kinetics of f- and b-Pheo (2×10^{-6} *M* in DMF); *T* = 300 K

	f-Pheo		b-Pheo	
λ_F (nm)	τ_i (ns)	α_i (%)	τ_i (ns)	α_i (%)
	1.28	36.2	2.12	56.3
680	5.64	63.2	5.30	43.7
	—	—	0.05	65.8
	2.61	44	1.94	19.2
730	6.04	56	5.46	15.0

the chromophores in b-Pheo is monomeric (M in Fig. 4). This is in agreement with the appearance of a weak aggregate band at ~ 730 nm in addition to the strong monomer emission at 670 nm in the room temperature fluorescence spectrum (Fig. 2). The intensity of the aggregate band has very little dependence on temperature, and is also found to be present in the 4.2 K fluorescence spectrum of solid b-Pheo in DMF. [In the pure solid b-Pheo, the aggregate band has shifted to ~ 740 nm and has an amplitude comparable to that of the monomer-emission occurring at 670 nm (data not shown).] If the chromatophores in b-Pheo form an approximately parallel dimer, simple exciton-theory predicts a low yield for fluorescence from the singlet exciton level of lowest energy, and an enhanced intersystem crossing consistent with the above-mentioned results. The essentials of this model are presented in Fig. 5 (right-hand side), identifying T_1^A as a triplet generated in the aggregate by singlet energy transfer from monomeric b-Pheo (M).

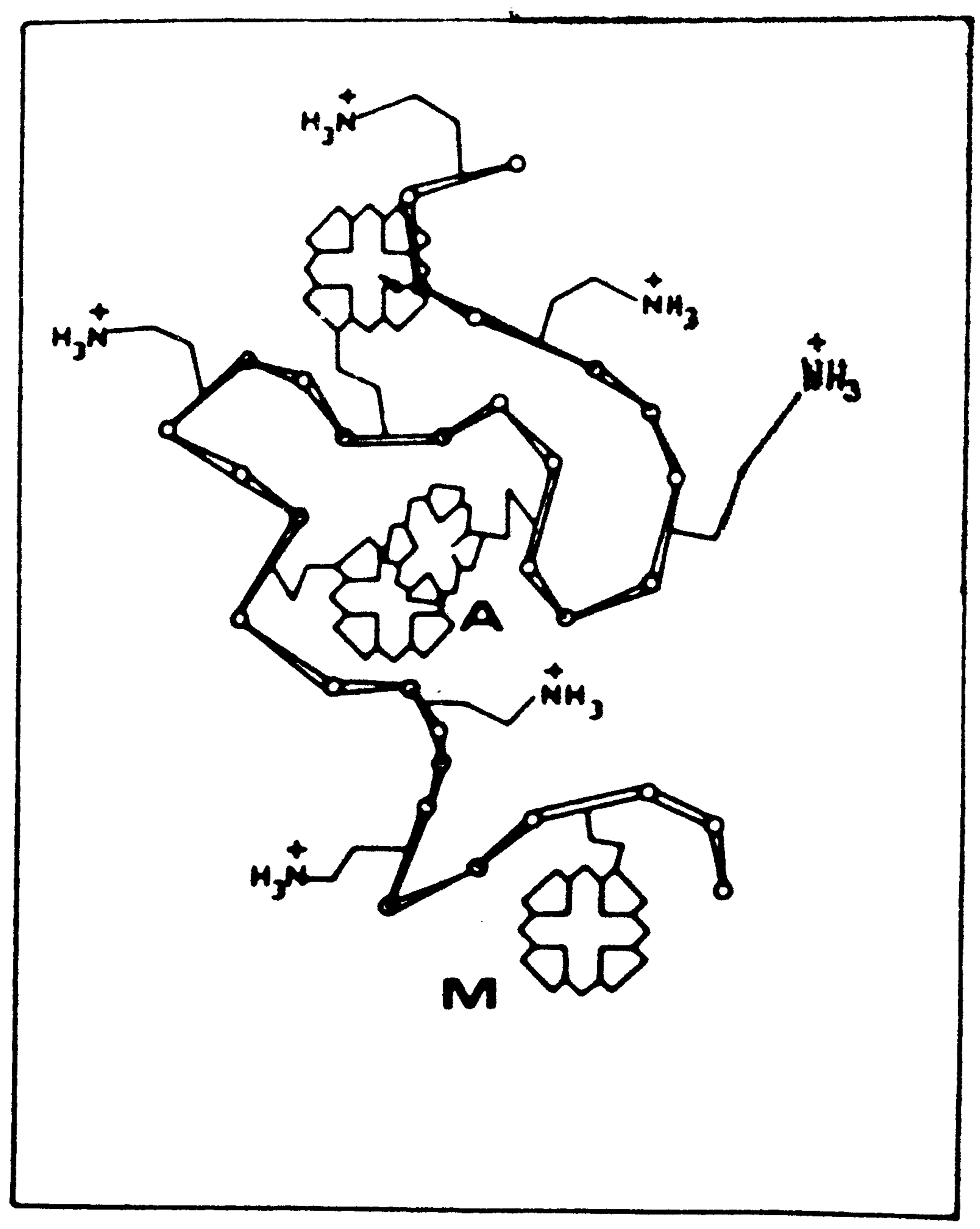

Figure 4. Proposed model of a polypeptide chain associated with monomeric (M) and aggregated (A) forms of b-Pheo.

Further support for this model comes from a comparison of the signs of FDMR transitions as observed experimentally and predicted, using the kinetic data obtained from FF experiments (Table 2). For f-Pheo the signs of the D−E and D+E transitions (see Fig. 3) imply that the ordering of the energies of T_1 spin-levels is E(i) > E(j) > E(k) or the other way around (Note, that i,j, and k label

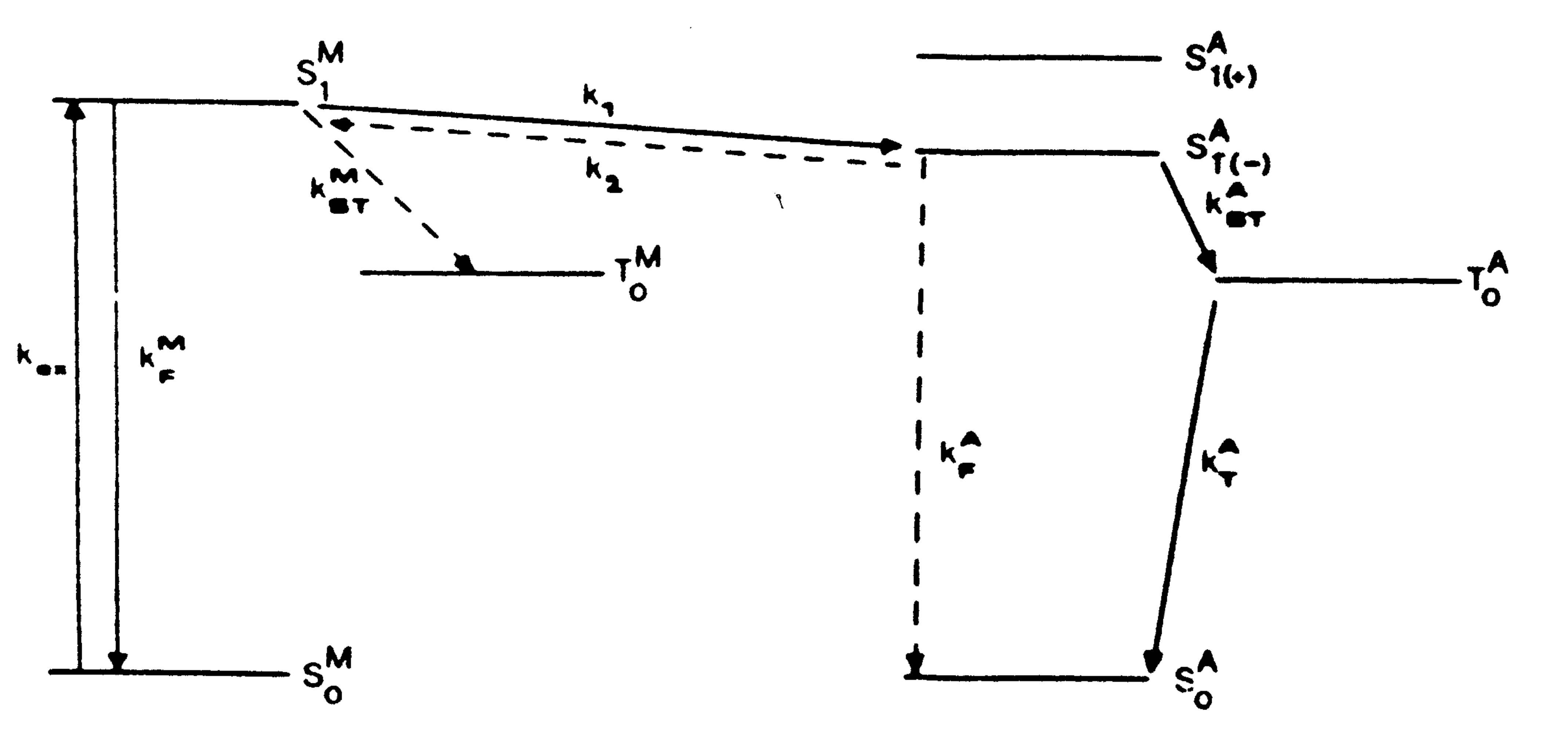

Figure 5. Kinetic scheme for singlet energy transfer M → A and intersystem crossing S_1^A → T_1^A in b-Pheo. M and A denote monomeric and aggregated pheophorbide molecules in b-Pheo, respectively. Fully drawn arrows represent processes with high yield, dashed arrows with zero or low yield. The center of gravity of the singlet exciton states $S_{1(+)}^{A}$, and $S^{A(-)}$ of the A-form is arbitrarily chosen to be at lower energy than the S_1^M state. The level-scheme of A represents a parallel, stacked Pheo dimer, of which the lower singlet exciton state $S_{1(-)}^A$, has an optically forbidden transition to the ground state.

two fast and one more slowly decaying component in FF curves). For porphyrins, it has been shown (Chan et al., 1971) that spin-level $|k>$ has the lowest energy and can be described as the zero-field spin-funtion $|z>$ for which $S_z|z> = 0$, where S_z represents the z-component of the triplet spin-operator. Analogously, the spin-levels $|i>$ and $|j>$ correspond to the spinfunctions $|x>$ and $|y>$ for which $S_x\ |x> = 0$ and $S_y\ |y> = 0$, respectively; (Van der Waals and de Groot, 1967); x, y and z denote two, mutually perpendicular in-plane axes of the Pheo molecule, z is the axis perpendicular to the molecular plane.

Taking $E(i=x) > E(j=y) > E(k=z)$ for the T_1 spin levels, the sign of the FDMR transitions determined by relation (3) is then negative for D−E transition ($|y> \longleftrightarrow |z>$) and positive for the D+E transition ($|x> \longleftrightarrow |z>$), as follows from

$$\Delta I_F = -A\,\frac{k_m - k_{m'}}{k_m + k_{m'}}\,(N_m - N_{m'}) \qquad (3)$$

where $m \neq m' = x,y,z$ and A is a positive parameter containing kinetic constants and the rate constant of excitation (Clarke and Hofeldt, 1974; Clarke, 1982; van der Bent et al., 1975). Equation 3 predicts the signs of both transitions correctly (Table 2) (Note, that for this calculation we have used experimental triplet state kinetic constants determined by FF at the same excitation intensity as in FDMR experiments).

For b-Pheo, the experimentally observed positive signs of D−E and D+E FDMR transitions are however inverted compared to those predicted from FF data for a free molecule (Table 2). This sign inversion can be explained by assuming that we are in fact observing the T_1^A triplet by monitoring fluorescence in the main 670–680 nm band of monomeric b-Pheo (M) within the folded polypeptide; b-Pheo (A) is excited by singlet-singlet energy transfer from b-Pheo (M). It can be shown (see Appendix) that under steady-state conditions, a change of the triplet population of the accepting trap by resonant microwave absorption, results in a change of monomer fluorescence with the *same* sign (Hoff and de Vries, 1981; G. H. van Brakel, The triplet state of chlorophyll-*a* in whole algal cells, Thesis, Agricultural University, Wageningen 1982; J. Beck, ODMR on pigment-complex of photosynthetic bacteria, University of Stuttgart, Stuttgart, 1983). For free molecules these changes are predicted to have *opposite* sign (Clarke and Hofeldt, 1974; van der Bent et al., 1975a).

The presence of singlet energy transfer from b-Pheo M to A, followed by intersystem crossing in A was also investigated by prompt fluorescence decay kinetics (FDK). As shown in Table 3, at 680 nm two nanosecond decay components were observed. The fluorescence of non-crystalline systems—e.g. pigment-polypeptides—can be simulated by a sum of exponentials. Energy transfer in these systems is expected to be non-coherent. Kenkre (1982a, 1982b, 1983) has shown that both S–S noncoherent energy transfer as well as fast S–T intersystem crossing lead to solutions of the Pauli master equations in exponential form. These effects produce in our case a shortening of the lifetimes of the experimental components in the fluorescence decay of b-Pheo compared to f-Pheo. We ascribe the 2.12 ns component to the lifetime of excited Pheo-monomers, shortened by energy transfer. Taking the 5.64 ns lifetime of f-Pheo to represent monomers incapable of singlet energy transfer, the bimolecular energy transfer rate constant $k_t = 3.2 \times 10^8\ [M]^{-1}\ s^{-1}$, where [M] is the concentration of monomers in the b-Pheo. Note, however, that f-Pheo also contains a shortened component with a lower, but still considerable amplitude as compared to b-Pheo. This indicates that in solid solutions also f-Pheo forms aggregates. Similar effects have been observed in pheophytin-*a* (van der Bent and Schaafsma, 1975b). At 730 nm where the aggregate-trap of b-Pheo emits weakly, the b-Pheo FDK contains a major (66%) ultrashort (~ 50 ps) component, which is absent at 680 nm. This component is assigned to the S_1-lifetime of the aggregate-trap, shortened by very fast intersystem crossing within the aggregate, resulting in a high yield of the triplet detected in b-Pheo using FDMR in the 670 nm emission-band. This explanation rests on assignment of the 730 nm fluorescence to aggregated b-Pheo, which is consistent with reported *in vitro* experiments on pheophytin-a (Kooyman et al., 1979).

We conclude that b-Pheo exhibits several significant differences with f-Pheo, using FDMR, FF and FDK techniques, which can be suitably explained by aggregation, induced by folding of the polypeptide, to which the pheophorbide is attached. The model proposed for b-Pheo provides an attractive basis for interpreting results obtained with the abovementioned methods for chlorophyll-proteins isolated from plants and bacteria, containing low-lying traps (e.g. active reaction centers).

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APPENDIX

Figure 5 describes a pigment-bed of N_M monomers (M) and N_A aggregates (A) acting as a trap. Singlet energy transfer may occur from M to A and *vice versa*, with rate constants k_1 and k_2, respectively. It is assumed that only monomers are excited (k_{ex}). All relevant decay processes are assumed to be first-order; their rate constants are defined in Fig. 5.

We wish to find the relation between a change of triplet population (ΔT_1^M and ΔT_1^A) due to resonant microwave transitions between the spin-levels of T_1^M or T_1^A, and the resulting change of fluorescence, i.e. the change in the excited singlet state populations ΔS_1^M or ΔS_1^A. The rate Eqs for this system can be solved under steady state conditions, assuming that singlet energy-transfer between pigment-bed and trap can be described by the bimolecular forward and backward transfer reactions

$$S_1^M + S_0^A \underset{k_1}{\overset{k_2}{\rightleftharpoons}} S_0^M + S_1^A$$

followed by intersystem crossing $S_1^A \xrightarrow{k_{ST}^A} T_1^A$. All above mentioned phenomena can be described in the set of four equations (A1).

$$\begin{aligned} & S_1^M + S_0^M + T_1^M = N_M \\ & S_1^A + S_0^A + T_1^A = N_A \\ & \dot{S}_1^M = S_0^M(k_{ex} + k_2 S_1^A) - S_1^M(k_F^M + k_{ST}^M + k_1 S_0^A) \\ & \dot{S}_1^A = S_0^A\, k_1\, S_1^M - S_1^A(k_F^A + k_{ST}^A + k_2 S_0^M) \end{aligned} \qquad \text{(A1)}$$

Under the steady state conditions, the change of pigment-bed fluorescence due to a change of triplet population of trap molecules is given by

$$\begin{aligned} \Delta I_F^M &= k_F^M\, \Delta S_1^M \\ &= k_{ex}\, \Phi_F^M\, \Phi_E (1 - \Phi_E)(1 - \Phi_{E'})(1 - \Phi_E \Phi_{E'})^{-2}\left(\frac{N_M}{N_A}\right)\Delta T_1^A \end{aligned} \qquad \text{(A2)}$$

where k_{ex} is excitation rate constant of monomeric pigment-bed molecules, ϕ_F^M, ϕ_F^A fluorescence yields in the absence of energy transfer, ϕ_E and $\phi_{E'}$, are forward and reverse energy transfer yields, respectively.

$$\Phi_F^M = \frac{k_F^M}{k_F^M + k_{ST}^M} \qquad \Phi_F^A = \frac{k_F^A}{k_F^A + k_{ST}^A}$$

$$\Phi_E = \frac{k_1 N_A}{k_F^M + k_{ST}^M + k_1 N_A} \qquad \Phi_{E'} = \frac{k_2 N_M}{k_F^A + k_{ST}^A + k_2 N_M}$$

Furthermore, it has been assumed that the fraction of bed- or trap-molecules in S_1 or T_1 is $\ll 1$. Equation (A2) predicts that in the presence of singlet-energy transfer ΔT_1^A and ΔI_F^M have the *same* sign. For $\Delta I_F^M(\Delta T_1^M)$, $\Delta I_F^A(\Delta T_1^M)$, and $\Delta I_F^A(\Delta T_1^A)$, the following expressions are obtained, now with a *negative* sign:

$$\Delta I_F^M = -k_{ex}\phi_F^M(1 - \phi_E)(1 - \phi_E\,\phi_{E'}^2)(1 - \phi_E\,\phi_{E'})^{-2}\,\Delta T_1^M \quad \text{(A3)}$$

$$\Delta I_F^M = -k_{ex}\phi_F^A\,\phi_E(1 - \phi_{E'})^2(1 - \phi_E\,\phi_{E'})^{-2}\,\Delta T_1^M \quad \text{(A4)}$$

$$\Delta I_F^A = -k_{ex}\Phi_F^A\Phi_E(1 - \Phi_E)(1 - \Phi_{E'})(1 - \Phi_E\Phi_{E'})^{-2}\left(\frac{N_M}{N_A}\right)\Delta T_1^A \quad \text{(A5)}$$

Note that the sign-inversion of ΔI_F^M (A2) holds as long as $\phi_E \neq 0$ or 1. Furthermore, the amplitude of FDMR spectra of the triplet trap, detected by pigment-bed fluorescence (Eqn. A2) is multiplied by a factor N_M/N_A, which usually is $\gg 1$. This factor is lacking in Eqs (A3) and (A4), but present in (A5). For the present model system, ϕ_F^A is low, however, due to fast intersystem crossing $S_1^A \rightarrow T_1^A$. This explains, why only FDMR signals of T_1^A are detected in the fluorescence of the monomer-bed.

For fluorescence-fading, the rate equation

$$\dot{S}_1^M = \dot{S}_0^A - (k_F^M + k_{ST}^M)\,S_1^M + k_{ex}S_0^M$$
$$= \dot{S}_0^A - (k_F^M + k_{ST}^M + k_{ex})\,S_1^M + k_{ex}\,N_M \quad \text{(A6)}$$

for S_1^M can be solved in the presence of bed-to-trap energy transfer, neglecting back-transfer and T_1^M population. For low excitation rate k_{ex} and, consequently, low population of S_1, the first term in (A6) is dominant at $t \gg (k_F^M + k_{ST}^M + k_{ex})^{-1}$, representing the decrease of ground-state traps (S_0^A) as a result of increasing T_1^A population. It is straightforward to show, that (A6) leads to

$$I_F^M(t) = I_F^M(O) + k_F^M \sum_{m=1}^{3} T_x^{A(m)}\,(e^{-k_m t} - 1) \quad \text{(A7)}$$

where k_m = decay rate constant of spin-level $|m\rangle$ of T_1^A, and $I_F^M(t)$ represents the FF curve; $T_x^{A(m)}$ denotes the steady state population of the spin-level $|m\rangle$ of the aggregate triplet state.