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# Simulation of ultrafast non-exponential fluorescence decay induced by electron transfer in FMN binding protein

Nadtanet Nunthaboot[a], Fumio Tanaka[a,*], Sirirat Kokpol[b,c], Haik Chosrowjan[d], Seiji Taniguchi[c], Noboru Mataga[c]

[a] Department of Chemistry, Faculty of Science, Mahasarakham University, Mahasarakham 44150, Thailand
[b] Department of Chemistry, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand
[c] Center of Excellence for Petroleum, Petrochemicals, and Advanced Materials, Chulalongkorn University, Bangkok 10330, Thailand
[d] Institute for Laser Technology, Utsubo-Hommachi 1-8-4, Nishiku, Osaka 550-0004, Japan

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ABSTRACT

The ultrafast non-exponential fluorescence decay of FMN binding protein (FBP) was analyzed with three electron transfer (ET) theories, Marcus theory, Bixon and Jortner theory and Kakitani and Mataga theory. Center to center distances between electron acceptor, the excited isoalloxazine, and donors, Trp-32, Tyr-35 and Trp-106, in FBP were determined by molecular dynamic simulation. Electron transfer parameters containing in these theories were determined so as to fit the calculated decay with the observed decay, according to a non-linear least squares method. Introduction of electrostatic energies between isoalloxazine anion and other ionic groups and between the donor cations and other ionic groups in the protein into any ET theories improved the fitting. The non-exponential behavior in the fluorescence decay is considered to be ascribed to a fluctuation of the protein structure with long period.



## 1. Introduction

Intense green fluorescence of free flavins in aqueous solution is almost completely quenched upon binding to many proteins. Even in these flavoproteins, however, fluorescence was observed [1–6] when they were excited with very short laser pulses. Tryptophan (Trp) and/or tyrosine (Tyr) always exist near isoalloxazine ring (Iso) in these flavoproteins with short emission lifetimes. It was demonstrated by means of transient absorption spectroscopy that photo-induced electron transfer (ET) takes place from these aromatic amino acids to the excited free Iso in solution [7] and in flavodoxins [8,9]. Fluorescence decays of the flavoproteins were always non-exponential. In some flavoproteins the slight deviation from the exponential decay was attributed to traces of free FMN [1–6]. However, the decays were still non-exponential after removing the contribution of the free flavins. Exact origin of the non-exponential behavior in the decay functions of bound flavins in flavoproteins has been remained unclear.

FMN binding protein (FBP) from *Desulfovibrio vulgaris*, strain Miyazaki, forms dimer in solution and in crystal phase. It binds one molecule of FMN per subunit. FBP is considered to play important role on electron transport among oxido-reduction proteins, but not identified yet [10]. The protein structure of the FBP in crystal form was determined by X-ray diffraction technique [11] while its structure in solution was obtained by NMR method [12]. Fluorescence decay fitting parameters of FBP in solution were 167 fs (96%) and 1.5 ps (4%), while 730 fs (60%) and 10 ps (40%) in single crystals [6]. The decays observed in both crystal and solution phases are non-exponential the present work, we have tried to elucidate the non-exponential behavior of the fluorescence decay of FBP in solution, by means of molecular dynamic simulation (MD) and ET theories.

## 2. Methods of analysis

### 2.1. MD simulation

MD simulation was carried out with time step of 2 fs using AMBER8 software package [13], starting from one of 20 NMR

* Corresponding author.
*E-mail address:* fukoh2003@yahoo.com (F. Tanaka).

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structures (PDB code 1AXJ) [12]. All missing hydrogen atoms of the protein were added using the LEaP module [13]. Force field parameters for the FMN were obtained from Schneider and Suhnel [14]. The system was solvated in a cubic box ($9 \times 9 \times 9\,nm^3$) with 6344 TIP3P water molecules, and set up under the isobaric-isothermal ensemble (NPT) with a constant pressure of 1 atm. and constant temperature of 298 K. Electrostatic interaction was corrected by the Particle Mesh Ewald method [15] and SHAKE algorithm [16] was employed to constrain all bonds containing hydrogen atoms. A cutoff distance was 1 nm for a non-bonded pair interaction. The atomic coordinates were collected from 1200 to 2200 ps at 2 ps time intervals.

### 2.2. ET theories

ET rates ($k_{ET}$) were calculated with the atomic coordinates obtained by MD and with three ET theories, according to Marcus [17–19], Bixon and Jortner [20–22] and Kakitani and Mataga [23–25]. These theories are summarized as follows:

Marcus (M) theory [17–19],

$$k_{ET} = \frac{2\pi}{\hbar} H_{ab}^2 \frac{\exp\{-\beta(R-\sigma)\}}{\sqrt{4\pi\lambda_s k_B T}} \exp\left\{-\frac{(\Delta G^0 - e^2/\varepsilon_0 R + \lambda_s + ES)}{4\lambda_s k_B T}\right\} \quad (1)$$

$H_{ab}$ is electronic interaction energy between donor and acceptor at donor–acceptor distance $R$ equal to $\sigma$, where $\sigma$ is a distance of van der Waals contact. $\exp\{-\beta(R-\sigma)\}$ expresses an electronic tunneling term where $\beta$ is a tunneling coefficient. $\Delta G^0$ is standard free energy gap between products and reactants. ES represents electrostatic energy described below. $\hbar$, $k_B$ and $T$ are Planck constant, Boltzmann constant and temperature, respectively. $\lambda_s$ is known as solvent reorganization energy [17] and expressed as:

$$\lambda_s = e^2\left(\frac{1}{2a_1} + \frac{1}{2a_2} - \frac{1}{R}\right)\left(\frac{1}{\varepsilon_\infty} - \frac{1}{\varepsilon_0}\right) \quad (2)$$

where $a_1$ and $a_2$ are radii of acceptor and donor when these reactants are assumed to be spherical, and $\varepsilon_\infty$ and $\varepsilon_0$ are optical and static dielectric constants. Optical dielectric constant used was 2.0, which was common in all theories. The radii of Iso, Trp and Tyr were determined in the following way: (1) three dimensional size of lumiflavin for Iso, 3-methylindole for Trp, and *p*-methylphenol for Tyr, were obtained by semi-empirical molecular orbital calculations (PM3), (2) the volumes of these molecules were determined as asymmetric rotors and (3) radii of spheres having the same volumes with the asymmetric rotors were obtained. The value of $a_1$ of Iso was 0.224 nm, and $a_2$'s for Trp and Tyr were 0.196 and 0.173 nm, respectively. Eq. (1) is called Marcus–Hush equation containing tunneling term [19].

Bixon and Jortner (BJ) theory [20–22]:

$$k_{ET} = \frac{2\pi}{\hbar} H_{ab}^2 \frac{\exp\{-\beta(R-\sigma) - S\}}{\sqrt{2\pi\lambda_s k_B T}} \sum_{i=0}^{n} \frac{S^i}{i!} \exp \times \left\{-\frac{(\Delta G^0 - e^2/\varepsilon_0 R + \lambda_s + i\hbar\langle\omega\rangle + ES)^2}{4\lambda_s k_B T}\right\} \quad (3)$$

$S = \lambda_V/\hbar\langle\omega\rangle$ is a vibronic coupling constant, where $\lambda_V$ is reorganization energy associated with the averaged frequency, $\hbar\langle\omega\rangle$. The value of $\sigma$ for Trp, $\sigma_{Trp}$ was approximated to be $\sigma_{Trp} = a_{Iso} + a_{Trp}$, where $a_{Iso}$ and $a_{Trp}$ are radii of Iso and Trp stated above, respectively. $\sigma_{Tyr}$ was expressed to be $\sigma_{Trp} = a_{Iso} + a_{Tyr}$, where $a_{Tyr}$ is radius of Tyr. $\sigma_{Trp}$ and $\sigma_{Tyr}$ were 0.42 nm and 0.397 nm, respectively. $n$ is number of vibronic coupling summation and was set to 9.

Kakitani and Mataga (KM) theory [23–25]:

$$k_{ET} = \frac{\nu_0}{1 + \exp\{\gamma(R - R_0)\}} \sqrt{\frac{k_B T}{4\pi\lambda_S}} \times \exp\left[-\frac{\{\Delta G^0 - e^2/\varepsilon_0 R + \lambda_S + ES\}^2}{4\lambda_S k_B T}\right] \quad (4)$$

In Eq. (4) $\nu_0$ is frequency and $\gamma$ is a coefficient related to ET process. The ET process is adiabatic, when $R < R_0$, and non-adiabatic when $R > R_0$. The other constants are common among Eqs. (1)–(4).

The standard free energy was expressed with ionization potential of ET donor, $E_{IP}$, as Eq. (5).

$$\Delta G^0 = E_{IP} - G^0_{Iso} \quad (5)$$

$G^0_{Iso}$ is standard Gibbs energy related to electron affinity of the excited Iso. $E'_{IP}$s of Trp and Tyr used for the analysis were 7.2 eV and 8.0 eV, respectively [26].

### 2.3. Electrostatic energy (ES) in protein.

Protein systems contain many ionic groups, which is different from systems in bulk solution. ES between $Iso^-$ and all ionic amino acid residues including phosphate anions of FMN [ES(Iso)], between $Trp\text{-}32^+$ and all ionic amino acid residues including phosphate anions of FMN [ES(Tyr-32)], between $Tyr\text{-}35^+$ and all ionic amino acid residues including phosphate anions of FMN [ES(Tyr-35)], and between $Trp\text{-}106^+$ and all ionic amino acid residues including phosphate anions of FMN [ES(Tyr-106)], are expressed as Eqs. (6)–(9), respectively.

$$ES(Iso) = \sum_{i=1}^{8} \frac{C_{Iso}C_{Glu}}{\varepsilon_0 R_{Iso}(Glu - i)} + \sum_{i=1}^{4} \frac{C_{Iso}C_{Asp}}{\varepsilon_0 R_{Iso}(Asp - i)} + \sum_{i=1}^{5} \frac{C_{Iso}C_{Lys}}{\varepsilon_0 R_{Iso}(Lys - i)} + \sum_{i=1}^{9} \frac{C_{Iso}C_{Arg}}{\varepsilon_0 R_{Iso}(Arg - i)} + \sum_{i=1}^{2} \frac{C_{Iso}C_{P}}{\varepsilon_0 R_{Iso}(P - i)} \quad (6)$$

$$ES(Trp - 32) = \sum_{i=1}^{8} \frac{C_{32}C_{Glu}}{\varepsilon_0 R_{32}(Glu - i)} + \sum_{i=1}^{4} \frac{C_{32}C_{Asp}}{\varepsilon_0 R_{32}(Asp - i)} + \sum_{i=1}^{5} \frac{C_{32}C_{Lys}}{\varepsilon_0 R_{32}(Lys - i)} + \sum_{i=1}^{9} \frac{C_{32}C_{Arg}}{\varepsilon_0 R_{32}(Arg - i)} + \sum_{i=1}^{2} \frac{C_{32}C_{P}}{\varepsilon_0 R_{32}(P - i)} \quad (7)$$

$$ES(Tyr - 35) = \sum_{i=1}^{8} \frac{C_{35}C_{Glu}}{\varepsilon_0 R_{35}(Glu - i)} + \sum_{i=1}^{4} \frac{C_{35}C_{Asp}}{\varepsilon_0 R_{35}(Asp - i)} + \sum_{i=1}^{5} \frac{C_{35}C_{Lys}}{\varepsilon_0 R_{35}(Lys - i)} + \sum_{i=1}^{9} \frac{C_{35}C_{Arg}}{\varepsilon_0 R_{35}(Arg - i)} + \sum_{i=1}^{2} \frac{C_{35}C_{P}}{\varepsilon_0 R_{35}(P - i)} \quad (8)$$

$$\mathrm{ES(Trp-106)} = \sum_{i=1}^{8} \frac{C_{106}C_{\mathrm{Glu}}}{\varepsilon_0 R_{106}(\mathrm{Glu}-i)} + \sum_{i=1}^{4} \frac{C_{106}C_{\mathrm{Asp}}}{\varepsilon_0 R_{106}(\mathrm{Asp}-i)} + \sum_{i=1}^{5} \frac{C_{106}C_{\mathrm{Lys}}}{\varepsilon_0 R_{106}(\mathrm{Lys}-i)} + \sum_{i=1}^{9} \frac{C_{106}C_{\mathrm{Arg}}}{\varepsilon_0 R_{106}(\mathrm{Arg}-i)} + \sum_{i=1}^{2} \frac{C_{106}C_{\mathrm{P}}}{\varepsilon_0 R_{106}(\mathrm{P}-i)} \quad (9)$$

In Eqs. (6)–(9), $C_{\mathrm{Iso}}$ is the charge of Iso anion and is equal to $-e$. $C_{32}$, $C_{35}$ and $C_{106}$ are the charges of Trp-32 cation, Tyr-35 cation and Trp-106 cation, respectively, and equal to $+e$. $C_{\mathrm{Glu}}$ and $C_{\mathrm{Asp}}$ are the charges of Glu and Asp in FBP, respectively, and equal to $-e$. $C_{\mathrm{Lys}}$ and $C_{\mathrm{Arg}}$ are the charges of Lys and Arg, respectively, and equal to $+e$. $C_{\mathrm{P}}$ is the charge of phosphate of FMN and equal to $-e$. FBP contains 8 Glu's, 4 Asp's, 5 Lys's, 9 Arg's and 2 negative charges at FMN phosphate. Distances between Iso and $i$th Glu ($i$ = 1–8) are denoted as $R_{\mathrm{Iso}}$(Glu-i). Distances between Trp-32 and $i$th Glu ($i$ = 1–8) are denoted as $R_{32}$(Glu-$i$), and so on.

ES's were expressed as follows:

For $k_{\mathrm{ET}}^{\mathrm{Trp-32}}(t')$ ES = ES(Iso) + ES(Trp − 32)

For $k_{\mathrm{ET}}^{\mathrm{Tyr-35}}(t')$ES = ES(Iso) + *ES*(Tyr − 35)

For $k_{\mathrm{ET}}^{\mathrm{Trp-106}}(t')$ ES = ES(Iso) + ES(Trp − 106)

### 2.4. Observed and calculated fluorescence decays

Observed fluorescence decay function was expressed by Eq. (10) [6].

$$F_{\mathrm{obs}}(t) = 0.96\exp\left(-\frac{t}{0.169}\right) + 0.04\exp\left(-\frac{t}{1.5}\right) \quad (10)$$

Lifetimes are expressed in ps unit. The calculated decay was expressed as Eq. (11) [27].

$$F_{\mathrm{calc}}(t) = \left\langle \exp -\{k_{\mathrm{ET}}^{\mathrm{Trp-32}}(t') + k_{\mathrm{ET}}^{\mathrm{Tyr-35}}(t') + k_{\mathrm{ET}}^{\mathrm{Trp-106}}(t')\}t \right\rangle_{\mathrm{AV}} \quad (11)$$

$k_{\mathrm{ET}}^{\mathrm{Trp\text{-}32}}(t')$, $k_{\mathrm{ET}}^{\mathrm{Tyr\text{-}35}}(t')$ and $k_{\mathrm{ET}}^{\mathrm{Trp\text{-}106}}(t')$ are ET rates from Trp-32, from Tyr-35 and from Trp-106, respectively, to the excited Iso, which are given by Eqs. (1) or (3) or (4) means averaging procedure of the $\langle \ldots \rangle_{\mathrm{AV}}$ exponential function in Eq. (11) over t′ up to 1 ns at 2 ps time intervals. In Eq. (11) we assumed that the decay function is exponential between $t = t'$ and $t = t' + 2$ ps.

### 2.5. Determination of ET parameters

The unknown ET parameters listed in Table 1 were determined to obtain the minimum value of $\mathrm{Dev}^2$ given by Eq. (12), by means of a non-linear least squares method according to Marquardt algorithm

$$\mathrm{Dev}^2 = \frac{1}{N}\sum_{i=1}^{N} \frac{\{F_{\mathrm{calc}}(t_i) - F_{\mathrm{obs}}(t_i)\}^2}{F_{\mathrm{obs}}(t_i)} \quad (12)$$

$N$ is number of data points and was 500. Deviations are expressed by Eq. (13).

$$\mathrm{Deviation}(t_i) = \frac{\{F_{\mathrm{calc}}(t_i) - F_{\mathrm{obs}}(t_i)\}}{\sqrt{F_{\mathrm{obs}}(t_i)}} \quad (13)$$

## 3. Results

### 3.1. Protein structure and its dynamics obtained by MD

Fig. 1 shows one of the snapshots of the protein structure at FMN binding site obtained by MD. Fig. 2 shows changes in distances (center to center distances) between Iso and Trp-32 (R32), between Iso and Tyr-35 (R35) and between Iso and Trp-106 (R106). R32 changes around 0.64 nm, R35 around 1.03 nm and R106 around 1.0 nm. R32 was the shortest throughout the time range (1 ns). The mean R32 over MD time ranges was 0.64 nm, the mean R35, 1.05 nm, and the mean R106, 0.99. These distances were compared with NMR distances [12], 0.84 nm between Iso and Trp-32, 0.74 nm between Iso and Tyr-35, and 0.82 nm between Iso and Trp-106. In crystal [11] these distances were 0.71, 0.77, and 0.85 nm, respectively. MD distance of R32 was quite shorter than those obtained by NMR and crystal structures, while MD distances of R35 and R106 were quite longer than those obtained by NMR and crystal structures.

### 3.2. Dynamics of electrostatic interaction inside the protein

Fig. 3 shows ES given by Eqs. (6)–(9). The value of $\varepsilon_0$ was 4.82 (see Table 1) which was determined by KM theory. Elso was 0.145 ± 0.001 eV, E32 0.023 ± 0.002 eV, E35 0.227 ± 0.003 eV, E35 0.154 ± 0.002 eV. ES was largest in E35, and lowest in E32. These

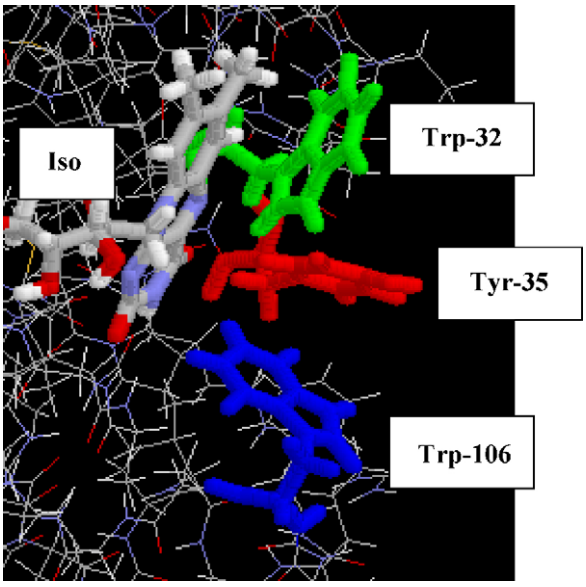


**Fig. 1.** Proteins structure of FBP at flavin binding site obtained by MD.

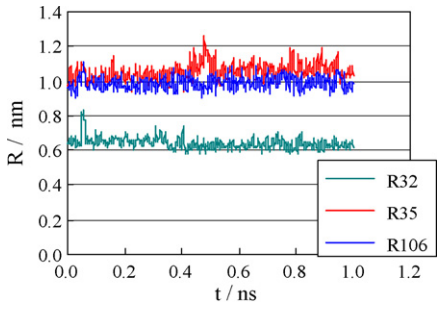


**Fig. 2.** Distances between Iso and nearby aromatic amino acids. R32, R35 and R106 are distances between Iso and Trp-32, Tyr-35 and Trp-106, respectively. The distances were obtained averaging over all distances between atoms in Iso and aromatic atoms in these amino acids.

**Table 1**
Best-fit ET parameter[a].

| Theory | ES[b] energy | Best-fit ET parameter | | | | | | Dev²[c] |
|---|---|---|---|---|---|---|---|---|
| M theory[d] | | $H_{ab}$ (eV) | $\beta$ (nm$^{-1}$) | $G^0_{Iso}$ (eV) | $\varepsilon_0$ | | | |
| | Neglected | 4.50 | 3.94 | 7.56 | 9.05 | | | $4.07 \times 10^{-4}$ |
| | Included | 0.383 | 19.2 | 7.05 | 2.72 | | | $1.83 \times 10^{-4}$ |
| BJ theory[d] | | $H_{ab}$ (eV) | $\beta$ (nm$^{-1}$) | $\lambda_V$ (eV) | $\hbar\langle\omega\rangle$ (eV) | $G^0_{Iso}$ (eV) | $\varepsilon_0$ | Dev²[c] |
| | Neglected | 1.48 | 10.5 | 0.588 | 0.0039 | 7.76 | 2.10 | $1.10 \times 10^{-3}$ |
| | Included | 0.978 | 8.99 | 0.834 | 0.0060 | 9.66 | 3.12 | $4.31 \times 10^{-4}$ |
| KM theory | | $\nu_0$ (ps$^{-1}$) | $\gamma$ (nm$^{-1}$) | $R_0$ (nm) | $G^0_{Iso}$ (eV) | $\varepsilon_0$ | | Dev²[c] |
| | Neglected | 661 | 28.3 | 1.08 | 9.71 | 4.44 | | $2.09 \times 10^{-4}$ |
| | Included | 297 | 31.1 | 1.12 | 9.62 | 4.82 | | $1.47 \times 10^{-4}$ |

[a] ET parameters are described by Eqs. (1), (3) and (4). Radii of Iso, Trp and Tyr were $a_{Iso}$ = 0.224 nm, $a_{Typ}$ = 0.196 nm and $a_{Tyr}$ = 0.173 nm, respectively. $\varepsilon_\infty$ = 2 was used. The observed fluorescence decay parameters of FBP were 0.167 ps (96%) and 1.5 ps (4%) and shown by Eq. (10) in the text.
[b] Electrostatic energy between Iso anion and all of ionic amino acids including phosphate anions, and between Trp or Tyr cation and all of ionic amino acids including phosphate anions, as shown by Eqs. (6)–(9) in the text.
[c] Dev² was obtained by Eq. (12).
[d] $\sigma_{Typ}$ = 0.42 nm and $\sigma_{Tyr}$ = 0.397 nm.

were mostly positive. Fluctuation of the energies was much more marked than one of the distances shown in Fig. 2.

### 3.3. Fluorescence decays

Ultrafast fluorescence decays of FBP are shown in Fig. 4. $F_{obs}(t)$ is given by Eq. (10). $F_{calc}(t)$ was calculated with the best-fit ET parameters obtained by M theory with the inclusion of ES. Upper panel of Fig. 4 shows the deviation between $F_{obs}(t)$ and $F_{calc}(t)$. Fig. 5 shows the fluorescence decays obtained by BJ theory with the inclusion of ES. Figs. 6 and 7 show the decays obtained by KM theory without and with inclusion of ES, respectively. In these figures the upper panels also show deviations between the observed and calculated decays. The agreement of $F_{calc}(t)$ with the observed non-exponential decay of FBP was quite well with the inclusion of ES, obtained by M theory and KM theory.

### 3.4. ET parameters

ET parameters in M theory, BJ theory and KM theory determined according to the method described above are listed in Table 1 without and with the inclusion of electrostatic energies. The values of Dev² with M theory were $4.07 \times 10^{-4}$ without ES and $1.83 \times 10^{-4}$ with ES. The values of Dev² with BJ theory were $5.79 \times 10^{-3}$ without ES and $2.45 \times 10^{-4}$ with ES. The values of Dev² with KM theory were $2.09 \times 10^{-4}$ without ES and $1.47 \times 10^{-4}$ with ES. Agreement between $F_{obs}(t)$ and $F_{calc}(t)$ improved by introducing the electrostatic energy in all theories. The value of Dev² between $F_{obs}(t)$ and $F_{calc}(t)$ was least with KM theory with inclusion of ES energy, though they did not differ much among three theories. KM theory also described well donor–acceptor distance-dependent rates of ET in flavoproteins [28]. In the previous work [28] original Marcus theory [17] was used, but in the present work Marcus–Hush equation containing the electronic tunneling term according to Moser et al. [19] was used as M theory. This equation improved very much the agreement between the observed and calculated fluorescence decays.

ET parameters obtained by M theory were $H_{ab}$ 0.385 eV, $\beta$ 19.2 nm$^{-1}$, $G^0_{Iso}$ 7.05 eV and $\varepsilon_0$ 2.72 with inclusion of ES. ET parameters obtained by BJ theory were $H_{ab}$ 0.978 eV, $\beta$ 8.99 nm$^{-1}$, $\lambda_V$

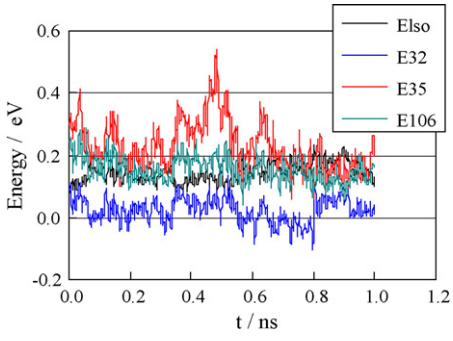


**Fig. 3.** Electrostatic energies of Iso anion and nearby aromatic amino acid cations in FBP. Elso indicates electrostatic energies between Iso anion and all ionic groups, E32 between Trp-32 cation and all ionic groups, E35 between Tyr-35 cation and all ionic groups, E106 between Trp-106 cation and all ionic groups. These were obtained with Eqs. (6)–(9) in text. $\varepsilon_0$ used for the calculation was 4.82 which was determined by KM theory.

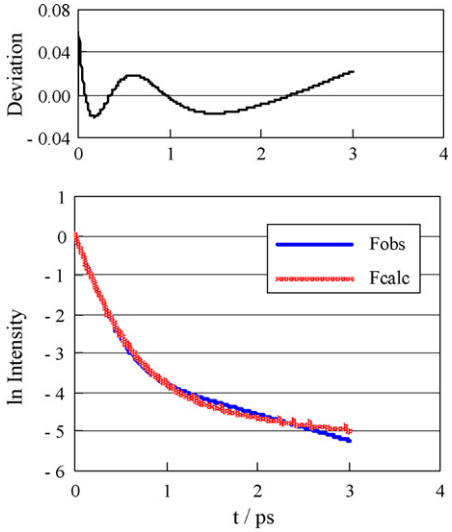


**Fig. 4.** Fluorescence decays of FBP obtained by M theory with the inclusion of ES. $F_{obs}$ and $F_{calc}$ indicate the observed and calculated fluorescence decays of FBP. $F_{calc}$ was obtained by M theory with ET parameters listed in Table 1. Upper panel shows the deviation between $F_{obs}$ and $F_{calc}$ according to Eq. (13). The value of Dev² given by Eq. (12) was $1.83 \times 10^{-4}$.

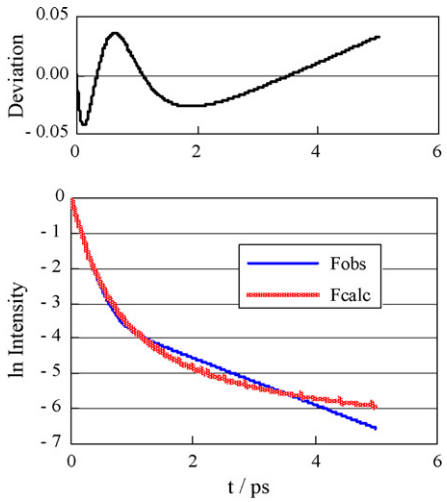


**Fig. 5.** Fluorescence decay obtained by BJ theory with the inclusion of ES. $F_{calc}$ was obtained by BJ theory with ET parameters listed in Table 1. Upper panel shows deviation between $F_{obs}$ and $F_{calc}$ obtained by Eq. (13). The value of $Dev^2$ given by Eq. (12) was $4.31 \times 10^{-4}$.

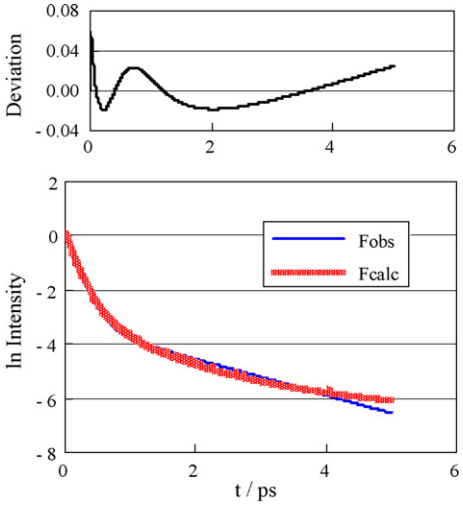


**Fig. 6.** Fluorescence decays of FBP obtained by KM theory without inclusion of ES. $F_{calc}$ denote the calculated fluorescence decays of FBP obtained by KM theory with ET parameters listed in Table 1. ES was not included in Eq. (4). The upper panel shows deviation between $F_{obs}$ and $F_{calc}$ obtained by Eq. (13). The value of $Dev^2$ given by Eq. (12) was $2.09 \times 10^{-4}$.

0.834 eV, $\hbar\langle\omega\rangle$ 0.006 eV, $G^0_{Iso}$ 9.66 eV and $\varepsilon_0$ 3.12, with the inclusion of ES. These parameters obtained by KM theory were $\nu_0$ 297 $ps^{-1}$, $\gamma$ 31.1 $nm^{-1}$, $R_0$ 1.12 nm, $G^0_{Iso}$ 9.62 eV and $\varepsilon_0$ 4.82 with the inclusion of ES.

## 4. Discussion

In the present work the ultrafast non-exponential fluorescence decay of FBP was analyzed using the donor–acceptor distances determined by MD, and ET theories. Most remarkable results of the present work are (1) fluorescence decay in the protein became non-exponential when we take average over much longer time range than the fluorescence decay, even though it is mono exponential at every instant, and (2) the introduction of electrostatic energy between ET products and other ionic groups enhanced the non-exponential behavior. Number of ET parameters were four by M theory, six by BJ theory and five by KM theory. These parameters were determined by the non-linear least squares method. The non-linear least squares method has been frequently used to determine unknown decay parameters. Number of unknown parameters, six, should not be too many to be determined accurately by the method. For example a decay function with three-lifetime components contains six parameters, which have been accurately determined by this method.

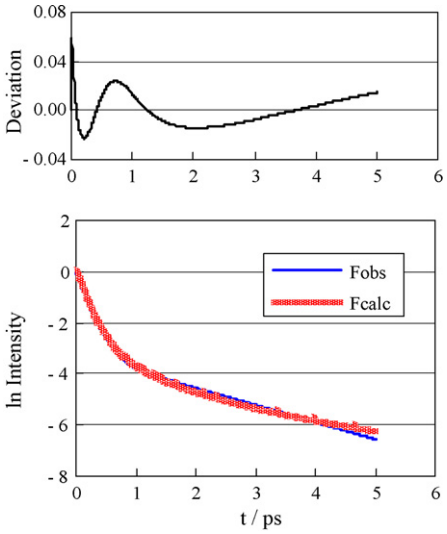


**Fig. 7.** Fluorescence decays of FBP obtained by KM theory with the inclusion of ES. $F_{calc}$ denotes the calculated fluorescence decay of FBP obtained by KM theory with the ET parameters listed in Table 1. ES was included in Eq. (4). The upper panel shows deviation between $F_{obs}$ and $F_{calc}$ obtained by Eq. (13). The value of $Dev^2$ given by Eq. (12) was $1.47 \times 10^{-4}$.

ET parameters obtained in the previous work [28] with KM theory were $\nu_0$ 636 $ps^{-1}$, $\gamma$ 65.2 $nm^{-1}$, $R_0$ 0.72 nm, $G^0_{Iso}$ 9.59 eV and $\varepsilon_0$ 3.20 in the analysis of donor–acceptor distance-dependent ET rates of 10 flavoproteins. Since in the work the average ET rates were used, the method of analysis may be static way. In the present work ET rate of FBP was analyzed from dynamic aspect. The ET parameters in KM theory obtained in the present work were $\nu_0$ 297 $ps^{-1}$, $\gamma$ 31.1 $nm^{-1}$, $R_0$ 1.12 nm, $G^0_{Iso}$ 9.62 eV and $\varepsilon_0$ 4.82. Though the value of $G^0_{Iso}$ was quite close between the two works, the values of $\nu_0$, $\gamma$ and $R_0$ in the present work were about half of the former result, and $\varepsilon_0$ in the present work was 1.5 times higher than the one in the previous work. Significance of the difference in the ET parameters between the results of dynamic and static analyses is not clear at the present. It should be noted that Eqs. (1), (3) and (4) for ET rate are for the static analysis. For dynamic analysis these may be modified as Sumi and Marcus for M theory [29].

When the decay was analyzed with the coordinates in rather narrow time range of 800–1000 ps (originally 2000–2200 ps) at time intervals of 10 fs, the non-exponential decay was never obtained with and without the inclusion of ES. Accordingly, it is important to take average in Eq. (7) over quite long time range compared to time range of fluorescence decay, to obtain the non-exponential decay of FBP. In other words, the structural fluctuation of protein with long period may be responsible for the non-exponential decay. The agreement between $F_{obs}(t)$ and $F_{calc}(t)$ with any ET theories improved, when the electrostatic energies were included in the ET rates. This suggests that the fluctuation of ES contributes to the non-exponential decay, and inclusion of the electrostatic energy to ET theories may be important for ET theories in proteins.

## 5. Conclusion

Ultrafast non-exponential fluorescence decay in FMN binding protein, which is induced by ET from aromatic amino acids to the excited FMN, was analyzed by means of the non-linear least squares method with ET theories and MD. Origin of the non-exponential decay was elucidated by long period fluctuation of protein structure. Electrostatic energies between anion of Iso and the other ionic groups, and between cation of Trp or Tyr and the other ionic groups may play important role on ET in the protein. The method may be applicable to ET processes in other flavoproteins and ET photoreceptors as well.

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