

Chapter 6 Hydration dynamics of BMVC in duplex and quadruplex DNA

6.1 Introduction

Telomeres, the end of chromosomes, has been extensively studied because its importance in biological systems.^{1,2,3,4} During replication, the length of telomeres decreased each cycle. Once the length of telomere shorten to certain extent, the cell stops replication and results in cellular senescence.^{5,6,7,8} In tumor cells, the length of telomere is maintained by telomerase, which leads to the abnormal overgrowth and immortalization of cancer cells.^{9,10,11} For above reason, the research about the interaction between telomere and telomerase has received large attention in past few years.^{12,13,14} In more than 85% of human tumor cells, the activity of telomerase increased significantly, and the inhibition of the activity of telomerase is thought to be

¹ Blackburn, E. H. and Greider, C. W. *Telomeres*; Cold Spring Harbor Laboratory press: New Work, 1996

² Blackburn, E. H. *Annu. Rev. Biochem.* **1984**, *53*, 163.

³ Williamson, J. R. *Annu. Rev. Biophys. Biomol. Struct.* **1994**, *23*, 703.

⁴ Colgin, L. and Reddel, R. *Current Biology* **2004**, *14*, R901.

⁵ Harley, C. B. and Futcher, A. B. *Nature* **1990**, *345*, 458.

⁶ Lundblad, V. and Szostak, J. W. *Cell* **1989**, *57*, 633.

⁷ Sandell, L. L. and Zakian, V. A. *Ibid.* **1993**, *75*, 729.

⁸ Harley, C. B. and Villeponteau, M. P. *Curr. Opin. Genet. Dev.* **1995**, *5*, 249.

⁹ Greider, C. W. and Blackburn, E. H. *Cell* **1987**, *51*, 887.

¹⁰ Feng, J., Funk, W. D., Wang, S.-S., Weinrich, S. L., Avilion, A. A., Chiu, C.-P., Adams, R. R., Chang, E., Allsopp, R. C., Yu, J., Le, S., West, M. D., Harley, C. B., Andrews, W. H., Greider, C. W. and Villeponteau, B. *Science* **1995**, *269*, 1236.

¹¹ Hahn, W. C., Counter, C. M., Lundberg, A. S., L., B. R., Brooks, M. W. and Weinberg, R. A. *Nature* **1999**, *400*, 464.

¹² Blackburn, E. H. *Ibid.* **1991**, *350*, 569.

¹³ Breslow, R. A., Shay, J. W., Gazdar, A. F. and Srivastava, S. J. *J. Natl. Cancer Inst.* **1997**, *89*, 618.

¹⁴ Mergny, J. L. and Hélène, C. *Nat. Med.* **1998**, *4*, 1366.

a potential cancer therapy.¹⁵ According to previous studies, the formation of quadruplex structure in telomeric 3'-overhang repeats could inhibit the activity of telomerase,^{16,17} and the interaction between telomerase and quadruplex structure has become more and more important. However, the existence of quadruplex structure in native human telomeres in vivo has not been verified and became a challenging question.¹⁸ In 2003, C.-C. Chang *et. al.* synthesized a novel fluorescence biomarker: 3,6-bis(1-methyl-4-vinylpyridinium) carbazole diiodide (BMVC), which can interact and recognize the linear duplex and quadruplex structures of DNA.¹⁹ With the application of BMVC, the existence of the quadruplex structure in native human telomeres was demonstrated for the first time.²⁰

In their studies, when BMVC is dissolved in tris-buffer, the fluorescence intensity is weak and central at ~590 nm. On the other word, when the molecule interacted with linear duplex and quadruplex DNA, the fluorescence intensity increased more than two order of magnitude and the emission band blue-shifted to ~560 and ~580 nm, respectively.²⁰ However, the studies of C. C. Chang *et. al.* are limited on steady state measurement and ns time-resolved emission spectra. With the application of femtosecond and picosecond time resolved fluorescence spectroscopy, the excited state dynamics of BMVC molecule has been investigated and reported in previous chapter. The result reveals that intramolecular motion would lead to an efficient non-radiative process. This process would be suppressed by the high viscosity of

¹⁵ Brunori, M., Luciano, P., Gilson, E. and Geli, V. *J. Mol. Med.* **2005**, *4*, 4.

¹⁶ Zahler, A. M., Williamson, J. R., Cech, T. R. and Prescott, D. M. *Nature* **1991**, *350*, 718.

¹⁷ Neidle, S. and Parkinson, G. N. *Nat. Rev. Drug Discovery* **2003**, *1*, 383.

¹⁸ Neidle, S. and Parkinson, G. N. *Curr. Opin. Struct. Biol.* **2003**, *13*, 275.

¹⁹ Chang, C.-C., Wu, J.-Y. and Chang, T.-C. *J. Chin. Chem. Soc.* **2003**, *50*, 185.

²⁰ Chang, C.-C., Kuo, I.-C., Ling, I.-F., Chen, C.-T., Chen, H.-C., Lou, P.-J., Lin, J.-J. and Chang, T.-C. *Anal. Chem.* **2004**, *76*, 4490.

glycerol. In this article, we focus on the specific interaction between BMVC and different types of DNA (linear duplex and quadruplex). In this studies, BMVC interacted with linear duplex DNA(LD) and Human quadruplex DNA(Hum 24). The result indicated that for BMVC/LD and BMVC/Hum24 complexes, BMVC is buried into a relative non-polar environment, and the restriction of intramolecular motion plays an important role in the fluorescence enhancement. Solvation dynamics are also investigated, we found that the solvation processes of BMVC in those two types of DNA are different, and the results would be discussed in following section.

6.2 Experimental section

BMVC molecules are synthesized and purified using the method that described elsewhere.¹⁹ The tris-buffer solution of 10 mM Tris-HCl ($\text{CH}_2(\text{OH})_3\text{CNH}_2$ PH 7.5) and 150 mM NaCl are prepared and used immediately. All oligonucleotides are purchased from Applied Biosystems. DNA samples are mixed with the tris-buffer solution and heated to 90°C for 5 mins. After heated, the solution cooled to room temperature and stored at 4°C more than 24 hours before using. The UV-Vis absorption spectra are recorded with CARY50 UV-Vis spectrophotometer (VARIAN). In emission spectra, excitation light source is frequency doubled femtosecond laser, and excitation wavelength fixed at 430 nm. The fluorescence is collected by an optical fiber and introduced into charge coupled device spectrometer (USB2000, Ocean optics). Picosecond temporally resolved spectra are obtained with a time-correlated single-photon counting (TCSPC) system (Fluotime 200, PicoQuant) as reported

previously.²¹ Femtosecond relaxation dynamics of the system are measured by fluorescence up-converted system (FOG100, CDP) in combine with a mode-locked Ti:sapphire laser as describe elsewhere.²²

6.3 Results and discussion

6.3.1 Steady state spectra

The steady state absorption and fluorescence spectra of BMVC in tris-buffer, LD and Hum24 are presented in Figure 6.1. The concentration of BMVC , LD and Hum 24 are fixed at 5 μ M. In tris-buffer, the absorption maximum located at 435nm. However, as BMVC interacted with LD and Hum24, the absorption shifted to 450nm and 470 nm, respectively. For π -conjugate system, the absorption wavelength increases with the increasing of conjugation length, and the planar structure would increase the effective conjugation length of the molecules.^{23,24} This result indicates that the ground state geometry of BMVC in Hum 24 is relative planar than that of LD and tris-buffer. In Figure 6.1, the emission spectra of BMVC in tris-buffer, LD, and Hum24 are indicated by dash line with different color. For BMVC in tris-buffer, the fluorescence of BMVC is weak and central at ~590 nm. When BMVC interacted with LD and Hum24, the fluorescence intensity increased more than two orders of magnitude, and this result has been reported by Chang *et. al.*^{19,25} On the other words,

²¹ Bhongale, C. J., Chang, C.-W., Lee, C.-S. and Diao, E. W.-G. *J. Phys. Chem. B* **2005**, *109*, 13472-13478.

²² Lu, Y.-C., Chang, C.-W. and Diao, E. W.-G. *J. Chin. Chem. Soc* **2002**, *49*, 693.

²³ Kuhn, H. and Försterling, H.-D. *Principles of physical chemistry*; Wiley: Chichester, 2000

²⁴ Atkins, P. and Jones, L. *Chemical principles: The quest for insight*, 3; W. H. Freeman and company: New York, 2004

²⁵ Chang, C.-C., Wu, J.-Y., Chien, C.-W., Wu, W.-S., Liu, H., Kang, C.-C., Yu, L.-J. and Chang, T.-C. *Anal. Chem.* **2003**, *75*, 6177.

we also observed the decreasing of the stoke shift when BMVC interacted with LD (4060 cm^{-1}) and Hum24 (3900 cm^{-1}). Similar results are also observed when BMVC dissolved in high viscosity solvent (glycerol). In high viscosity solvent, the molecule can not relax to the minimum of S_1 state because the restriction of intramolecular motion, and the fluorescence was emitted from the non-relax state. Therefore we observed the decreasing of the stoke-shift. Since we observed similar decreasing of the stoke-shift, this result supported that in BMVC/LD and BMVC/Hum24 complex, the intramolecular motion is also restricted.

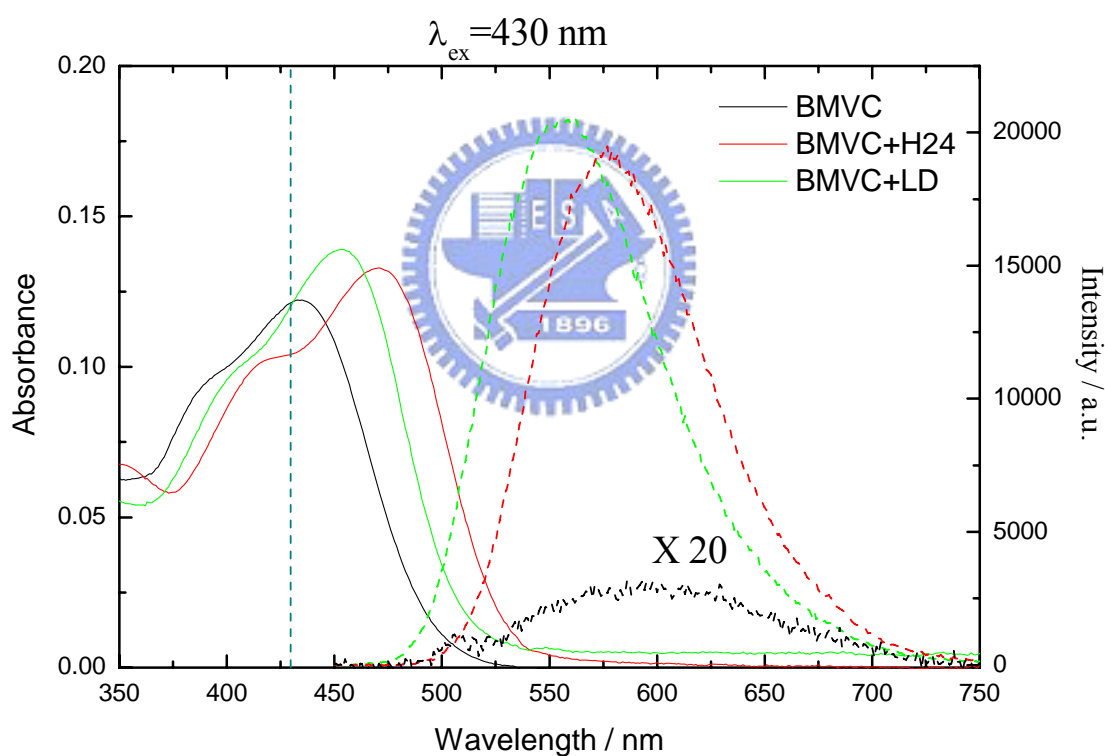


Figure 6.1: Steady state spectrum of BMVC and BMVC interacted with LD and Hum24 DNA. The concentration is fixed at 5×10^{-6} M, and the excitation wavelength was indicated as gray dash line.

6.3.2 Time-resolved fluorescence measurement

The time resolved fluorescence of BMVC in tris-buffer has been reported in

previous section. In high viscosity solvent (glycerol), the fluorescence lifetime increases due to the restriction of intramolecular motion. For comparison, similar measurement was performed as BMVC interacted with LD and Hum24 DNA. Figure 6.2 is the typical fluorescence transients of BMVC interacted with LD and Hum24 DNA. For comparison, the transient of BMVC in tris-buffer is also demonstrated. It is evident that the fluorescence lifetime increases significant due to the interaction between BMVC and DNA macromolecule. The time-resolved measurement supported that the interaction between BMVC and DNA would restrict the intramolecular motion, and caused the increasing of the fluorescence lifetime.

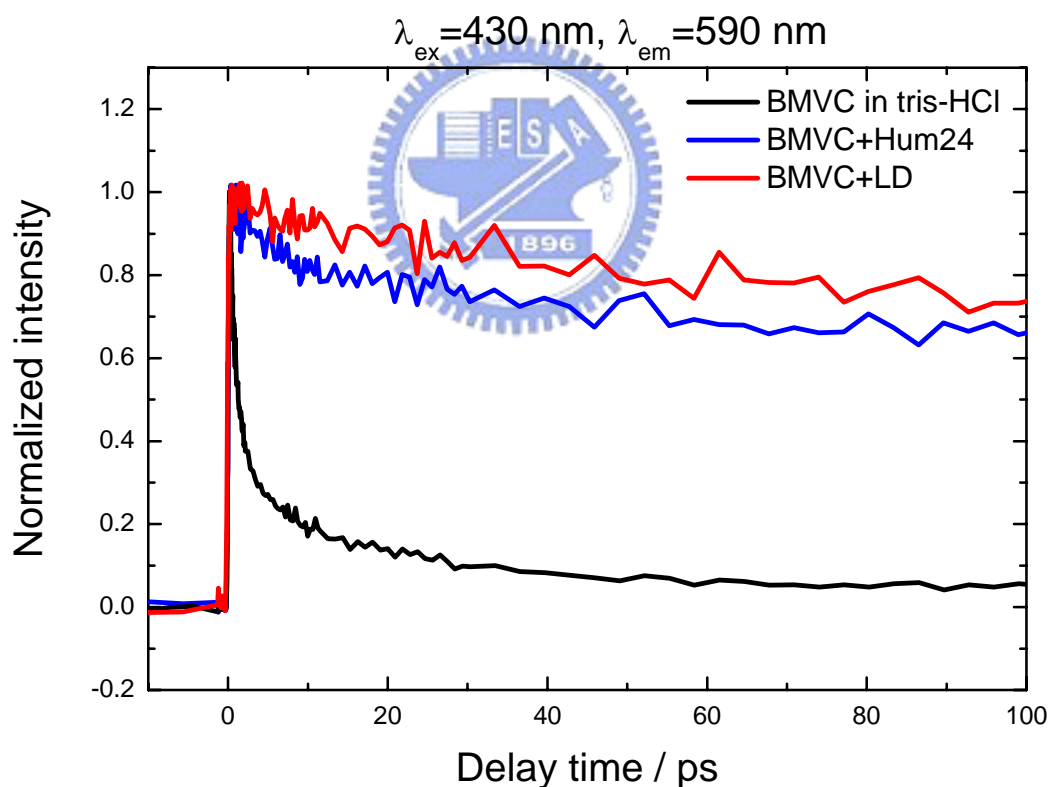
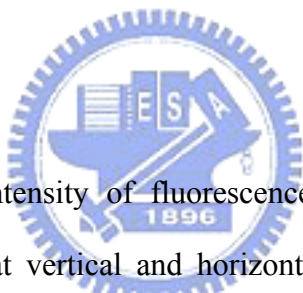


Figure 6.2: The femtosecond time-resolved spectra of BMVC in tris-buffer, and BMVC interacted with LD and Hum24 DNA. The excitation was fixed at 430 nm, and the probing wavelength was fixed at 590 nm

6.3.3 Fluorescence anisotropy measurement

When BMVC is mixed with LD and Hum 24, another important issue is the percentage of BMVC interacted with DNA. A useful tool is the temporally-resolved fluorescence anisotropy measurement, which can observe the rotational Brownian motion of the chromophore.^{26,27} According to Perrin's equation, the rotation diffusion coefficient is inversed proportional to the hydrodynamic molecular volume of fluorophore.²⁶ Therefore, the anisotropy decay of interact and non-interact molecule is quite different. In TCSPC experiment, the anisotropy decay can be calculated with the following equation:

$$r(t) = \frac{I_{VV}(t) - G \times I_{VH}(t)}{I_{VV}(t) + 2 \times G \times I_{VH}(t)} \quad (1)$$

 I_{VV} and I_{VH} represent the intensity of fluorescence which excite with vertically polarized light and monitor at vertical and horizontal polarization, respectively. G indicates the G-factor of monochromator. Figure 6.3 is the results obtained by TCSPC technique. In Figure 6.3A, the anisotropy decay of BMVC in tris-buffer can be fitted with a 0.37 ns decay. However, we also observed a long component (11.2 ns). Because the fluorescence decay of BMVC in tris-buffer is not long enough for us to observe the 11.2 ns anisotropy decay, the 11.2 ns component might be caused by the artificial effect. Figure 6.3B and Figure 6.3C are the fluorescence anisotropy decay of BMVC+Hum24 and BMVC+LD, respectively. In those two Figures, the anisotropy decay increases from 370 ps to 4.4 ns (Hum24) and 5.3 ns (LD). This result indicates

²⁶ Valeur, B. *Molecular Fluorescence: principles and applications*; WILEY-VCH: New York, 2002

²⁷ Lakowicz, J. R. *Principles of fluorescence spectroscopy*; Kluwer Academic/Plenum Publishers: New York, 1999

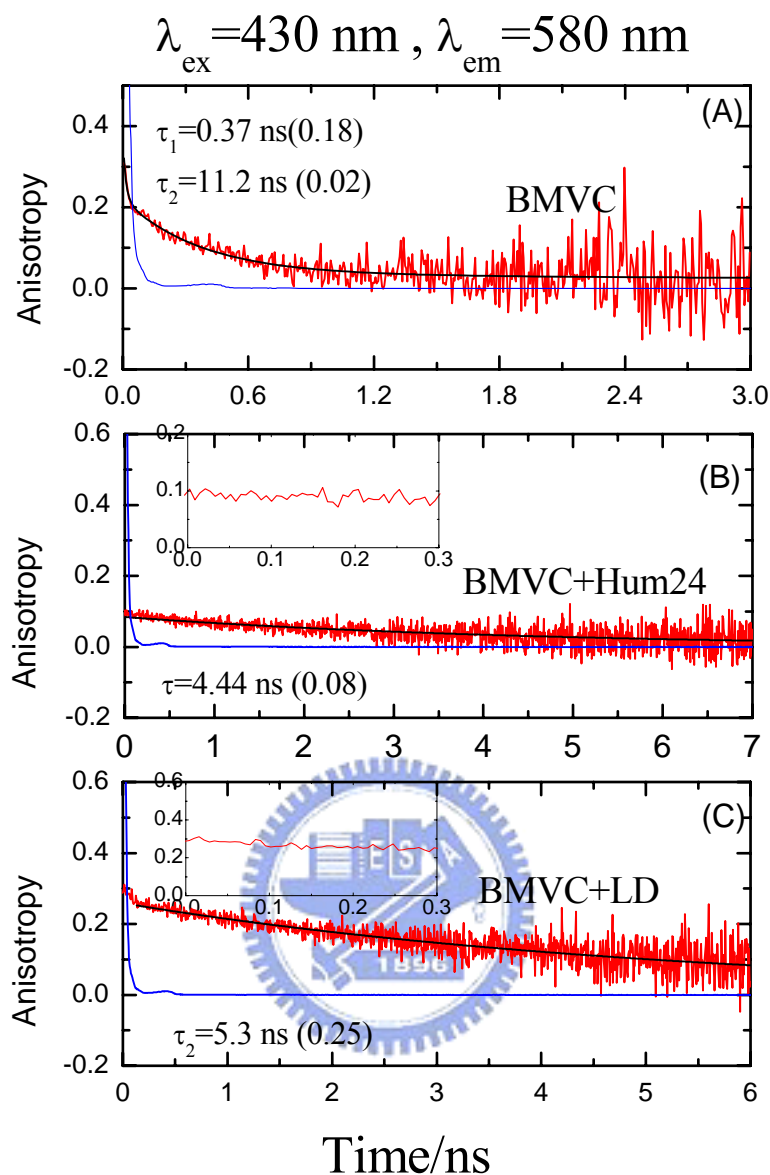


Figure 6.3: Fluorescence anisotropy decay of BMVC at various environments. The anisotropy was reconstructed using the TCSPC results, and the instrument response function is indicated as blue line. (A) BMVC in tris-buffer; (B) BMVC+Hum24; (C) BMVC+LD

that BMVC is connected with the DNA macromolecules, and the increase of the anisotropy time is attributed to the increase of the hydrodynamic volume.

Similar anisotropy measurement is also performed using fluorescence up-conversion technique. For up-conversion experiment, $r(t)$ can be calculated with the following equation:

$$r(t) = \frac{I_{//}(t) - I_{\perp}(t)}{I_{//}(t) + 2I_{\perp}(t)} \quad (2)$$

The polarization of the probe beam is fixed at horizontal, $I_{//}$ and I_{\perp} indicated the relative polarization of excitation beam at parallel and perpendicular direction, respectively.

In Figure 6.4A, the anisotropy decay can be fitted with a bi-exponential function. The fast component is 32 ps and the other one is fixed at the value obtained from TCSPC. Because not only the rotational Brownian motion but also the segmental motion would cause the depolarization of fluorescence, the 32 ps component is attributed to the segmental motion of molecules. However, in the anisotropy decay of BMVC/LD and BMVC/Hum24 complexes we do not observe any fast component in the range between 0~300 ps. This result indicates that the segmental motion of BMVC is restricted due to the interaction between BMVC and DNA and the amount of non-interact BMVC molecules is undetectable.

6.3.4 Hydration dynamics of BMVC/LD complex

Figure 6.5A is the typical fluorescence transients of BMVC/LD complex. The excitation wavelength is fixed at 430 nm and probed at 540 nm, 600 nm, and 640 nm. On the blue edge of spectra the signal decays with multi-exponential components at different time scale, whereas on the red edge of spectra a significant rise component is observed. In order to reconstruct the time-resolved emission spectra (TRES), we measured the transient with 10 nm interval in the range between 520 nm and 640 nm.

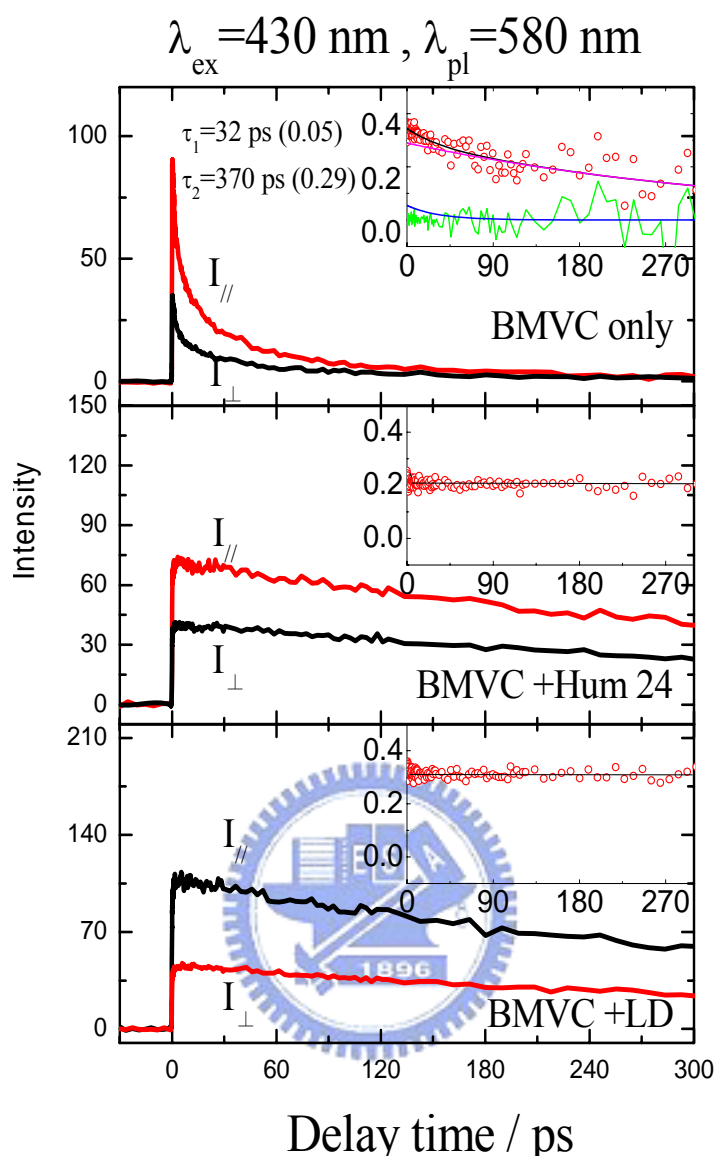


Figure 6.4: Femtosecond time-resolved anisotropy decay of (A) BMVC in tris-buffer, (B) BMVC/Hum24 and (C) BMVC/LD complex.

The reconstructed TRES are shown in Figure 6.5B, and the net spectral shift is 881 cm^{-1} within 50 ps. This result is smaller than that in tris-buffer ($\sim 2300 \text{ cm}^{-1}$). Similar decrease of the stoke shift in dye/DNA system is also observed in previous studies.^{28,29} This result indicated that in BMVC/LD complex, BMVC molecules are located in relative nonpolar environment in comparison with that in tris-buffer. Figure

²⁸ Pal, S. K., Zhao, L. and Zewail, A. H. *Proc. Natl. Acad. Sci.* **2003**, *100*, 8113.

²⁹ Brauns, E. B., Murphy, C. J. and Berg, M. A. *J. Am. Chem. Soc.* **1998**, *120*, 2449.

6.5C is the corresponding $C(t)$ function, which can be fitted by a bi-exponential decay function with time coefficients of 0.9 ps (0.88) and 8.4 ps (0.12).

BMVC+LD, $\lambda_{\text{ex}}=430 \text{ nm}$

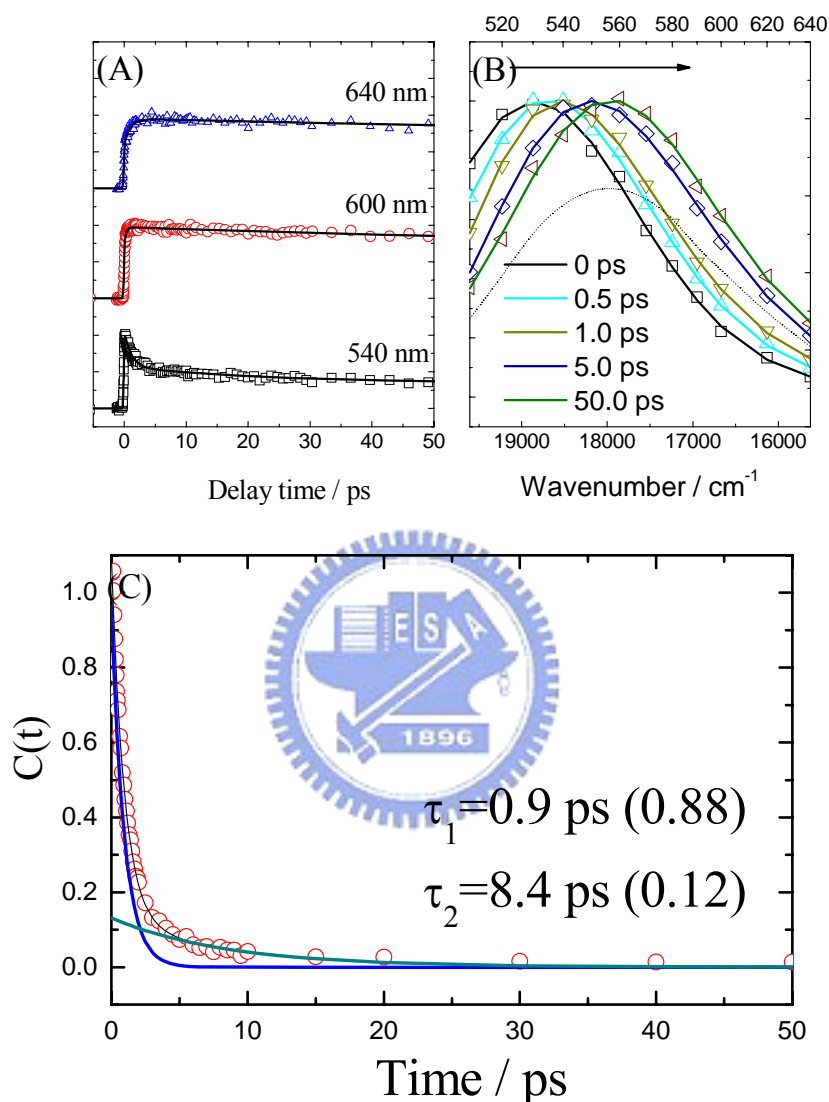


Figure 6.5: (A) The fluorescence transient of BMVC/ LD complex probing at 540 nm, 600nm, and 640 nm. (B) The constructed time-resolved emission spectrum of BMVC/ LD complex at various delay time. (C) The corresponding solvent relaxation spectra of BMVC/ LD complex

In 2003, Zewail group investigated the hydration dynamics of drug in the minor groove of duplex DNA.^{28,30} A bi-model hydration behavior was observed, and the results indicated that there are two kinds of water at DNA surfaces: bulk like (labile

³⁰ Pal, S. K., Zhao, L., Xia, T. and Zewail, A. H. *Proc. Natl. Acad. Sci.* **2003**, *100*, 13746.

water) and weakly bound (order water) water. Therefore, we believe that the 0.9 ps component is contributed from the bulk like water, and the 8.4 ps is attributed to the hydration caused by weakly bound water. However, according to previous study, the hydration process in the minor groove of DNA is determined to be ~ 20 ps,^{28,30} and the observing 8.4 ps process is significant shorter than that. This result indicated that in LD surface, the water close to BMVC has more degree of freedom than that in DNA minor grooves. The possible conformation is within the interface between major and minor groove, thus the effect of bound water is not as strong as that in minor groove.

6.3.5 Hydration dynamics of BMVC/Hum 24 complex

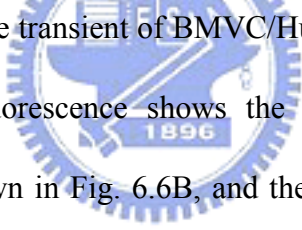


Figure 6.6A is the fluorescence transient of BMVC/Hum24 complex. Like the case of BMVC/LD complex, the fluorescence shows the typical solvation feature. The reconstructed spectra are shown in Fig. 6.6B, and the total spectral shift is 923 cm^{-1} within 50 ps. The decreasing of the stoke shift is similar to that of BMVC/LD complex, and we think that BMVC is also buried in the hydrophobic site of Hum24 DNA. The $C(t)$ function in Fig. 6.6C can be described with a bi-exponential function, and the time coefficients are 1.0 ps (0.6) and 9.5 ps (0.4). The time-scale of those two components is similar to that of BMVC/LD complex, and we believed that they are contributed from the bulk like water and weak bound water. However, the relative amplitude of the weak bound water (9.5 ps) is significantly larger than that in BMVC/LD DNA. The increasing of the relative amplitude of the weak bound water indicates that in BMVC/Hum24 complex BMVC feels more weak bound water,

therefore it might further buried into the DNA surface. The variety of the solvation dynamics of BMVC in LD and Hum24 indicated that it serve as a alternative method to recognize the DNA structure

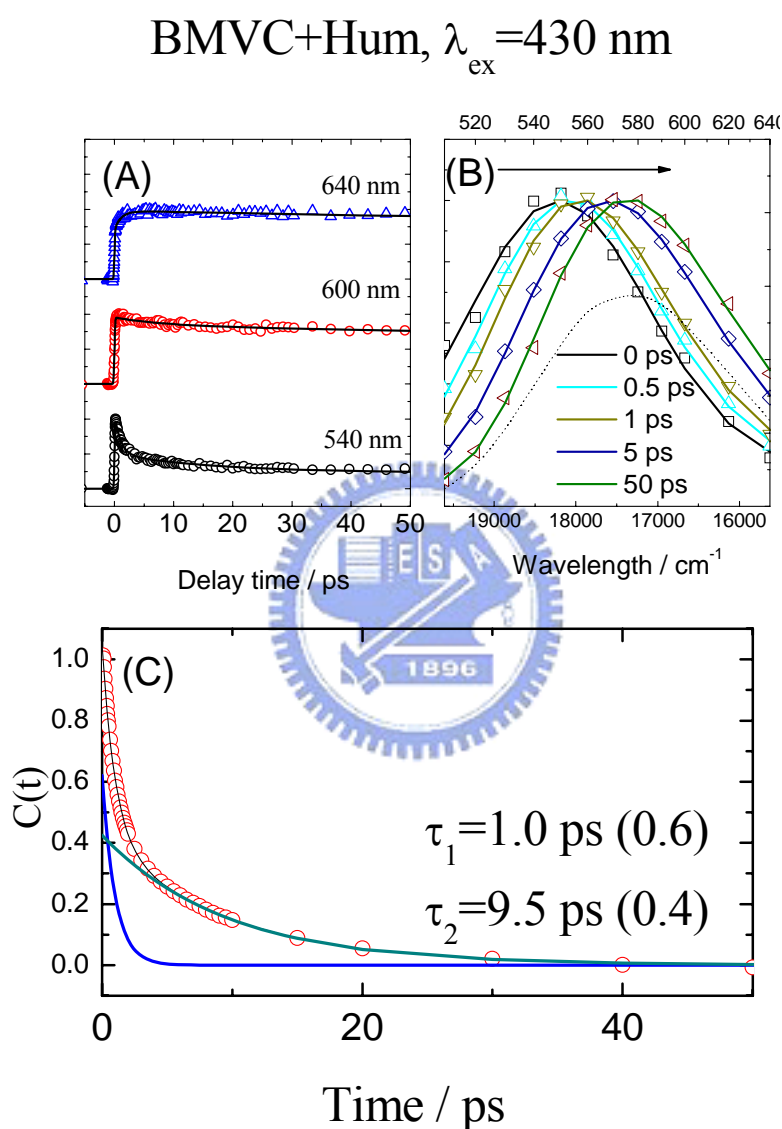


Figure 6.6: (A) The fluorescence transient of BMVC/ Hum24 complex probing at 540 nm, 600nm, and 640 nm. (B) The constructed time-resolved emission spectrum of BMVC/ Hum24 complex at various delay time. (C) The corresponding solvent relaxation spectra of BMVC/ Hum 24 complex

6.4 Concluding Remarks

In this study, we reported the fluorescence enhancement of BMVC/DNA (LD and Hum24) complex. The anisotropy measurement indicates that the segmental motion of BMVC in those two complexes is suppressed. Because similar fluorescence enhancements are also observed for BMVC in high viscosity solvent (glycerol), we concluded that the fluorescence enhancement in BMVC/LD and BMVC/Hum24 complexes is due to the restriction of intramolecular motion. The solvation dynamics of BMVC/DNA complex has also been investigated. For BMVC/LD complex, the $C(t)$ decay with two time coefficient: 0.9 ps and 8.4 ps. The 0.9 ps component is contributed from the solvation process of bulk like water, and the 8.4 ps is attributed to the solvation of weakly bound water. This result indicates that the binding site is located in the interface between major and minor groove. For BMVC/Hum24 complex, the solvation dynamics is similar to that of BMVC/LD complex. However, the relative amplitude of the weakly bound water is significantly larger than that of BMVC/LD complex, which indicated that BMVC is buried in the relative hydrophobic site of the DNA macromolecule.