

## The solvent effects on the energy gap between the $\pi\pi^*$ state and $\pi\pi^*$ state and fluorescence of benzoyleneurea

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**Abstract.** Benzoyleneurea (BenzU), a fluorescent nucleobase analog, exhibits strong solvent dependent fluorescence intensity. With the increasing of the concentration of polar and protic solvents, BenzU exhibits enhanced fluorescence. To understand the photophysical properties of BenzU, the energy gaps ( $\Delta E$ ) between dark  $\pi\pi^*$  state and bright  $\pi\pi^*$  state of BenzU are calculated in several solvents. The solvent dielectric constant and hydrogen bond between BenzU and protic solvent both have effects on  $\Delta E$ . And the fluorescence quantum yields of BenzU are linear response to  $\Delta E$ . Furthermore, we find that the more hydrogen bonds are formed between BenzU and protic solvent, the higher  $\Delta E$  and fluorescence intensity are obtained.

**Keywords:** Fluorescence quantum yields; Hydrogen bonds; Solvent effects;

### Introduction

The photo-initiated processes in modified nucleobases are discussed intensively in the past decades.[1-3] People are concerned about how modifying the structure affects the behavior of the excited state of bases molecules.[4-5] A more practical motivation is being able to obtain fluorescence emissive nucleobase analogs. Fluorescence is a sensitive and versatile technique for studying nucleic acid dynamics which is preferred over other techniques.[6] The nature nucleobases have very small fluorescence quantum yields associated with ultrashort excited state lifetimes.[7-9] Replacing the nature nucleobases with fluorescent nucleobase analogs can be used as fluorescent probes to study the interaction of nucleic acids with other biomolecules. Some of the fluorescent nucleobase analogs were used to explore environmental features of DNA/RNA. [10-11]

The fluorescence quantum yields of fluorophores are frequently affected by environmental solvents. [12] The factors that affect fluorescence emission spectra include solvent viscosity, [13-14] polarity, [15-17] and specific solvent and fluorophore interaction[17-20]. The specific effects can due to hydrogen bonding, charge-transfer interactions, etc.[17-19] It was reported that hydrogen bonding enhanced the fluorescence emission and induced larger Stokes shift. [17, 21]

It is well known that Nucleobases, uracil and thymine, have two lowest singlet excited state, dark  $\pi\pi^*$  state and bright  $\pi\pi^*$  state. [8, 22-26] It was found that water (or hydrogen bonding) and substituents have effects on  $\pi\pi^*$  state and  $\pi\pi^*$  state of DNA/RNA bases.[4-5,26-32] However, there are seldom reports focusing on solvent effects on the excited state dynamics of fluorescent nucleobases analogs.

Herein, we report the photophysical properties of benzoyleneurea (hereafter BenzU, **Figure 1**) in several solvents

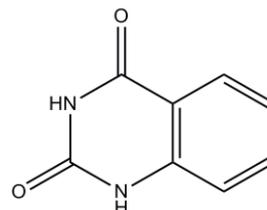
and solvent mixtures. BenzU is a uracil derivative. It provides 4  $\pi$ -electrons to uracil forming a large delocalization. The fluorescence quantum yields of BenzU are sensitive to the solvent polarity and hydrogen bond. BenzU may be a good candidate as an environmental probe. In order to elucidate the excited state dynamics of BenzU, the energy gaps between  $\pi\pi^*$  state and  $\pi\pi^*$  state of BenzU were calculated using time-dependent density functional theory (TD-DFT) methods.

### Experimental section

Absorption spectra were obtained using a PerkinElmer Lambda 35 double-beam spectrometer. The fluorescence spectra were recorded on a Horiba Jobin Yvon FluoroMax-4P spectrofluorometer. The fluorescence lifetime were probed by using time-correlated single photon counting (TCSPC) method. A 295 nm NanoLED (1.4 ns FWHM) was used as pulse laser source. The fluorescence lifetime were recorded at 365 nm fluorescence emission.

The benzoyleneurea (BenzU) was purchased from Tokyo Chemical Industry (Shanghai). It was used without further purification. The concentrations of BenzU in solvents were about to  $10^{-6}$  M. All the solvents used here were analytic degree and were purchased from J&K Chemical or Sinopharm chemical Reagent.

### Computational section



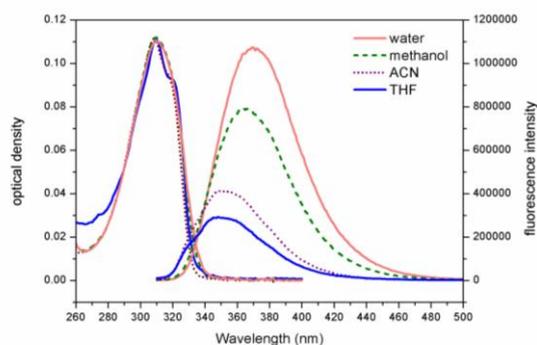
**Figure 1:** Molecular structure of benzoyleneurea.

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Density functional theory (DFT) and time-dependent DFT Calculations were performed using Gaussian 09 programs. [33] The equilibrium geometries of the ground state were optimized using DFT method. The calculations considered the solvent effects to be performed in the presence of a solvent by placing the solute in a cavity within the solvent reaction field

(SCRF). The polarizable continuum model (PCM) using the integral equation formalism variant is used in SCRF. The B3LYP functional and the 6-311+G (d,p) basis set were used for all calculations. The vertical excited energies with linear-response (LR) were calculated at optimized geometries using TD-DFT method. TD-DFT was also used to optimize the equilibrium geometries of the  $\pi\pi^*$  state and  $n\pi^*$  state. After the geometry optimization, the frequency analysis was performed.

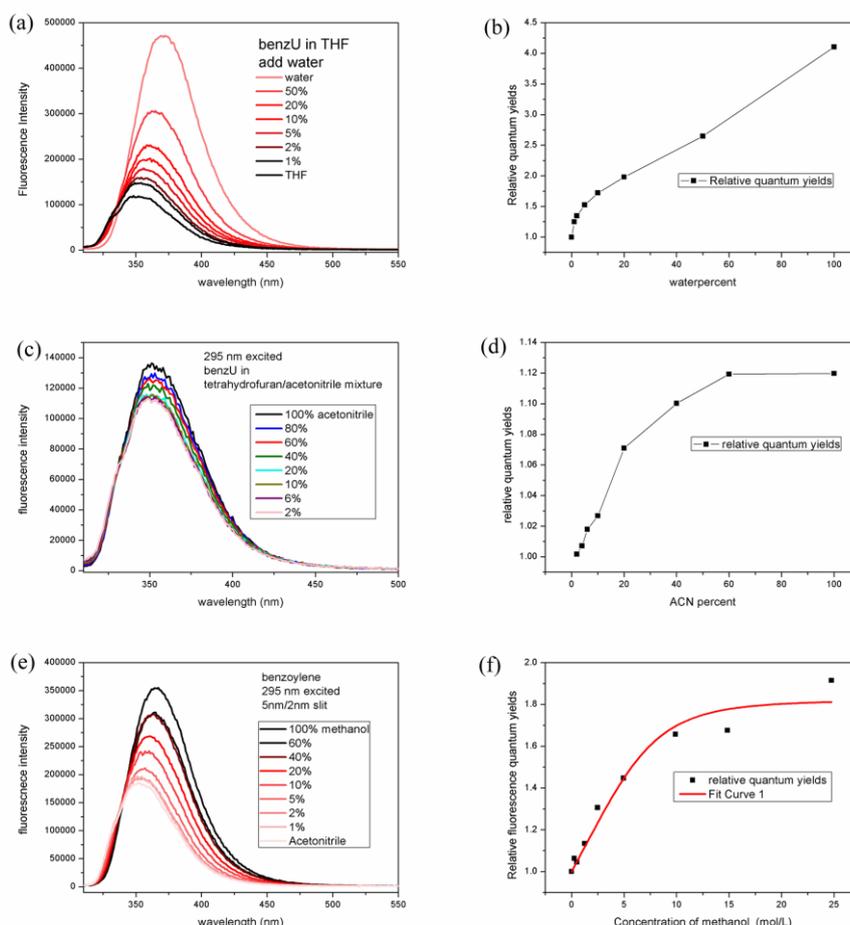
The potential energy profiles of excited states of BenzU in gas phase were checked with CC2 type calculations for better discussion. [34] These CC2 calculations were carried with the TURBOMOLE program suite, [35] making use of the resolution-of-the-identity (RI) approximation for the evaluation of the electron-repulsion integrals. The cc-pVTZ basis set has been employed in RI-CC2 calculations.



**Figure 2:** The absorption and fluorescence spectra of BenzU in 5 solvents.

## Results and Discussion

The absorption spectra of BenzU in 5 solvents are shown in **Figure 2**. It shows BenzU has vibrational structure in non-protic solvents, acetonitrile (MeCN), tetrahydrofuran (THF) and 1,4-dioxane (DOX). While in protic solvents, water and

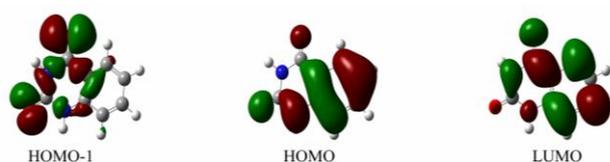


**Figure 3:** (a) Fluorescence of BenzU in THF/water mixture, (b) The fluorescence quantum yields of BenzU in THF/water mixture with increase of water concentration, (c) Fluorescence of BenzU in THF/MeCN mixture, (d) The fluorescence quantum yields of BenzU in THF/MeCN mixture with increase of water concentration, (e) Fluorescence of BenzU in MeCN/methanol mixture, (f) The fluorescence quantum yields of BenzU in MeCN/methanol mixture with increase of methanol concentration, the solid red line was fitted line using eq.3. The fluorescence quantum yields of BenzU at percent zero were set to 1 in (c), (e) and (f).

methanol (MeOH), this vibrational structure is disappeared and the spectra have a little red shift. This means that hydrogen bond complexes are formed in ground state (GS) between BenzU and protic solvents. [6,36] The fluorescence spectra of BenzU in several solvents are also exhibited in **Figure 2**. Obviously, the fluorescence intensity of BenzU is enhanced with the increasing of the solvent polarity and hydrogen bond donor. The Stokes shifts are similar in DOX, THF and MeCN. In protic solvents, the Stokes shifts are larger (15~20 nm) than in non-protic solvents. The enhanced fluorescence of BenzU in polar and protic solvents makes BenzU be a candidate to detect the properties of microenvironment.

The fluorescence dependent on the solvent polarity and hydrogen bond donor will be discussed in the following. **Figure 3** gives the fluorescence spectra of BenzU in three solvent mixtures, water/THF, MeCN/THF and MeCN/MeOH. The concentrations of one component in the mixtures are varied gradually from 0 to 100 percent. **Figure 3a** shows the fluorescence of BenzU in water/THF mixture. The fluorescence quantum yields used here are relative value to the fluorescence quantum yields of BenzU/THF solvent. **Figure 3b** exhibits that the fluorescence quantum yields of BenzU are increased with response to the adding of water to THF solution. However, it is non-linear increasing of fluorescence to the concentration of water. This non-linear response is difficult to understand. Actually, THF/water solvent mixture has two solvent effects, the general polar effect and specific hydrogen bond effect. We assume that the mixture of the two effects induces the non-linear response.

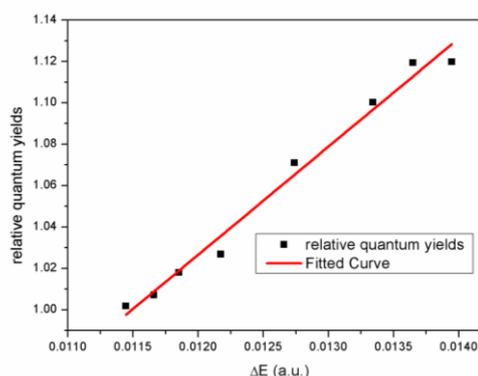
In order to distinguish the general polar effect and specific hydrogen bond effect, the fluorescence spectra of



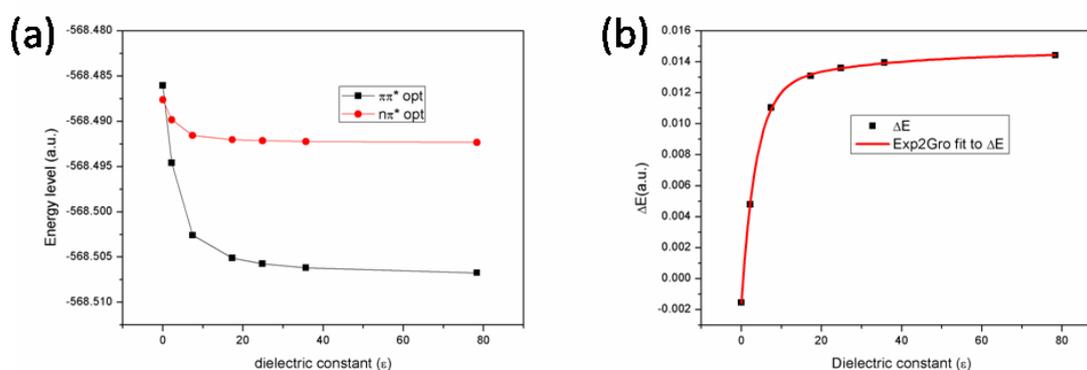
**Figure 4:** The frontier molecular orbitals were mentioned in this paper.

BenzU in MeCN/THF mixture and MeCN/MeOH mixture are measured (see **Figure 3c-3f**). In THF/MeCN mixture, the dielectric constant of MeCN is great larger than THF and there is no hydrogen bond effect. It is suitable used to study the solvent polar effect. In MeCN/MeOH mixture, the dielectric constants of MeCN and MeOH are very close and only hydrogen bonding effect should be considered. In THF/MeCN mixture, with increasing of the concentration of MeCN, the fluorescence of BenzU are increased and are exhibited a little Stokes shift (**Figure 3c**). However, non-linear response of fluorescence quantum yields to the concentration of MeCN is shown (**Figure 3d**). As well as, the fluorescence quantum yields of BenzU in MeCN/MeOH mixture are non-linear response to the concentration of MeOH too (**Figure 3e-3f**). So, single effect cannot obtain linear increase of fluorescence quantum yields. Another mechanism should be provided to understand the non-linear response of fluorescence. Furthermore, the solvent effects on excited states should be elucidated to better understand these non-linear responses in **Figure 3**.

Theoretical calculations were performed. The ground state (GS) optimization geometry was obtained in gas phase at B3LYP/6-311+g (d, p) level. And vertical excited energies (VEEs) were calculated for the lowest singlet states at GS optimization (see **Table 1**). The highest occupied molecular orbital (HOMO),



**Figure 6:** The plot of  $\Delta E$  to the fluorescence quantum yields of BenzU in THF/ACN solvent mixtures, the solid red line is fitted by linear function.



**Figure 5:** (a) The plot of the energy level of  $n\pi^*$  and  $\pi\pi^*$  states to dielectric constant, (b) The plot of energy gaps ( $\Delta E$ ) between  $n\pi^*$  and  $\pi\pi^*$  states to dielectric constant, the solid red curve is the bi-exponential function fitted curve.

lowest unoccupied molecular orbital (LUMO) and HOMO-1 are shown in **Figure 4**. After analysis of the transit orbitals and oscillator strengths, it was found that the first singlet excited state (S1) is a bright  $\pi\pi^*$  state and the second state (S2) is a dark  $n\pi^*$  state. Then,  $\pi\pi^*$  state and  $n\pi^*$  state were optimized separately at TD-B3LYP/6-311+g (d, p) level in gas phase, the VEEs at the each optimized geometries are also given in table 1. The Cartesian coordinates of GS,  $\pi\pi^*$  state and  $n\pi^*$  state optimized geometries are presented in the Supporting Information (see Table S1-S3). In **Table 1**, the energy levels of  $n\pi^*$  state and  $\pi\pi^*$  state are very close at GS optimized geometry. It is well known that  $n\pi^*$  state will influence the emission of  $\pi\pi^*$  state when their energy levels are proximity. [37] The energy gap between  $n\pi^*$  state and  $\pi\pi^*$  state is solvent sensitive, and the higher polarity of solvent will obtain larger energy gap.

Thus, solvent effects were considered in the calculations of optimized  $n\pi^*$  and  $\pi\pi^*$  state of BenzU. With the increasing of solvent dielectric constant, the energy of optimized  $n\pi^*$  state and  $\pi\pi^*$  state are decreased (**Figure 5a**). Generally, the lower energy gap will induce larger non-radiation rate from  $\pi\pi^*$  state to GS state according to Fermi's Golden Rule. Then, lower fluorescence quantum yields should be obtained. However, BenzU exhibits stronger fluorescence with increasing

of polarity of solvent environment. This contrast may be induced by decoupling of  $n\pi^*$  state and  $\pi\pi^*$  state. The energy gap between  $n\pi^*$  and  $\pi\pi^*$  state should be considered, which is the key parameter to measure the coupling of  $n\pi^*$  state and  $\pi\pi^*$  state. [6] **Figure 5b** shows obviously that the energy gap ( $\Delta E$ ) between  $n\pi^*$  and  $\pi\pi^*$  state enlarged with the increasing of solvent dielectric constant ( $\epsilon$ ). In particular, the energy gap shows a non-linear response to the dielectric constant ( $\epsilon$ ). A bi-exponential grown function was used to fit the data in **Figure 5b**. The fitted function is given at following:

$$\Delta E = 0.0145 - 0.0023 \exp\left(-\frac{\epsilon}{28.626}\right) - 0.0138 \exp\left(-\frac{\epsilon}{3.6717}\right) \quad (\text{eq.1})$$

Where,  $\Delta E$  is the energy gap; and  $\epsilon$  is the dielectric constant.

In MeCN/THF solvent mixture, the non-linear response of fluorescence quantum yields to the concentration of MeCN can be fitted using a bi-exponential function also. We assume there is a linear relationship between the fluorescence quantum yields and solvent dielectric constants. The dielectric constants of MeCN/THF mixture are calculated using the following equation: [7-8]

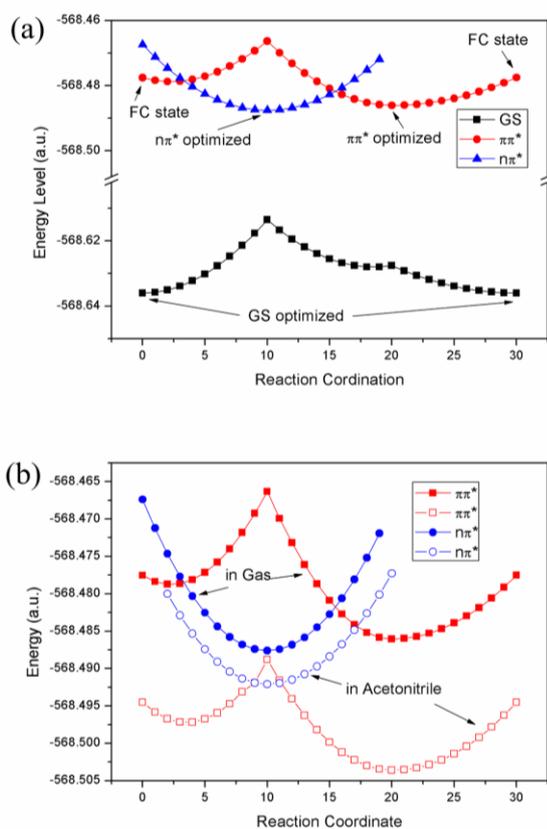
$$\epsilon_{\text{mix}} = \phi_1 \epsilon_1 + (1 - \phi_1) \epsilon_2 \quad (\text{eq.2})$$

Where,  $\phi_1$  is the volume fractions of solvent MeCN.

Then, the energy gaps in solvent mixture were calculated using the fitted bi-exponential function (eq. 1). Finally, the fluorescence quantum yields of BenzU in MeCN/THF mixture are plotted to the energy gaps in **Figure 6**, a linear response is obtained.

Furthermore, the excited state dynamics of BenzU are studied to clarify the effects of energy gap ( $\Delta E$ ) on the fluorescence intensity. The linearly interpolated in internal coordinates (LIIC) are performed from GS optimized geometry to  $n\pi^*$  state optimized geometry in gas phase using TD-DFT method. Then, the LIIC from  $n\pi^*$  state optimized geometry to  $\pi\pi^*$  state optimized geometry and the LIIC from  $\pi\pi^*$  state optimized geometry back to GS optimized geometry are calculated. Potential energy profiles of GS,  $\pi\pi^*$  state and  $n\pi^*$  state along the LIIC are given in **Figure 7a** (solid symbols). The same LIICs are also calculated at RI-CC2/cc-pVTZ level. **Figure S1** (Supporting Information) shows the results of RI-CC2 are similar to TD-DFT results. It means TD-DFT method is reliable. As shown in **Figure 7a**, there are two decay pathways for the Franck-Condon (FC) state populations. One is to  $n\pi^*$  state, another is to  $\pi\pi^*$  state. The populations on  $\pi\pi^*$  state and  $n\pi^*$  state may establish an equilibrium by conquer an energy barrier. We know that the fluorescence are come from the populations on  $\pi\pi^*$  state. Obviously, the populations will be increased when the energy level of  $\pi\pi^*$  state is decreased relative to  $n\pi^*$  state. Then, enhanced fluorescence should be obtained. Now, the solvent polarity is considered in calculation of LIIC. **Figure 7b** shows the LIIC (hollow symbols) calculated in MeCN by using IEFPCM model. It shows that  $n\pi^*$  state in MeCN has a little lower than in gas phase,  $\pi\pi^*$  state has a big lower than in gas phase. Therefore, the enhanced fluorescence in larger polar solvents should be understood. In gas phase or low polarity solvents, the excited state populations will be distributed on  $n\pi^*$  state greater than on  $\pi\pi^*$  state. But in high polarity solvents, populations are prefer distributed on  $\pi\pi^*$  state rather than on  $n\pi^*$  state.

Besides the general solvent polar effects, the fluorescence of BenzU also shows a non-linear response to the increasing of fractions of protic solvents (**Figure 3f**). The hydrogen bond complexes may play a key role. Form the UV-

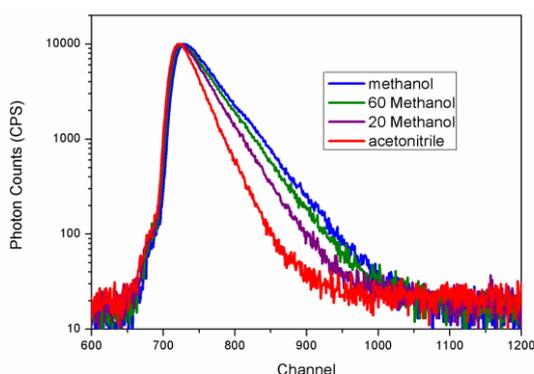


**Figure 7:** (a) Potential energy profiles of GS,  $n\pi^*$  and  $\pi\pi^*$  state in gas phase for the LIIC paths from GS optimized geometry to  $n\pi^*$  optimized geometry, to  $\pi\pi^*$  optimized geometry, back to GS optimized geometry. Where, FC state means Franck-Condon state. (b) Compared the LIIC potential energy profiles of  $n\pi^*$  and  $\pi\pi^*$  state in gas phase (solid square and circle) and in ACN (hollow square and circle).

Vis spectra of BenzU in **Figure 2**, it is deduced that hydrogen bond complex is formed at ground state in protic solvents. The fluorescence quantum yields and Stokes shifts of BenzU in THF/water and MeCN/MeOH mixtures exhibit a gradient increasing with increasing of methanol (see **Figure 3**). Furthermore, **Figure 8** shows the fluorescence lifetimes of BenzU in MeCN/MeOH mixture are also observed a gradient increasing as the addition of MeOH. Generally, two lifetimes should be obtained if there are two different lifetime fluorophores in solution. The gradient increasing of fluorescence lifetime means that there are many kinds of hydrogen complexes in MeCN/MeOH solvent mixture. **Figure S2** (Supporting Information) shows there are four hydrogen bonding sites in BenzU, two carbonyls C=O and two N-H bonds. And four hydrogen bonds at most are formed between BenzU and solvent molecules. Some of GS optimized geometries of the BenzU-MeOH complexes are calculated using TD-DFT method. And the VEEs from those GS optimized geometries are given in Table S4 (Supporting Information). The four mono-methanol complexes give different energy gap. And the more hydrogen bonds are formed between BenzU and MeOH, the higher energy gap between excited state S1 and S2 will obtain. Obviously, the number of hydrogen bonds is related to the concentration of protic solvent in solvent mixtures. Then, with the addition of protic solvent, the energy gap between S1 and S2 are increased. According to **Figure 5d**, the larger energy gap will obtain higher fluorescence. Therefore, enhanced fluorescence of BenzU is observed as adding protic solvent into non-protic solvent.

## Conclusions

In this paper, we reported the photophysical properties of BenzU in several solvents and solvent mixtures. BenzU shows enhanced fluorescence with increasing of polarity of solvent or special hydrogen bonding effect. It was found that the fluorescence of BenzU is a non-linear response to polar or protic solvent concentrations in solvent mixture. TD-DFT methods were used to calculate the energy of GS,  $\pi\pi^*$  and  $\pi\pi^*$  states. Solvent polar effects were considered in IEFPCM model. It was found that  $\Delta E$  between  $\pi\pi^*$  and  $\pi\pi^*$  states are increased with the increasing of solvent dielectric constant, whereas, the energy of  $\pi\pi^*$  and  $\pi\pi^*$  state are decreased with the increasing of solvent dielectric constant. A linear response of fluorescence quantum yields of BenzU to  $\Delta E$  was found in MeCN/THF solvent mixtures. The potential energy profiles of



**Figure 8:** Fluorescence lifetime decays of BenzU in MeCN/MeOH solvent mixture.

$\pi\pi^*$  and  $\pi\pi^*$  on LIIC paths were calculated. It was found that the excited state populations are prefer distributed on  $\pi\pi^*$  state rather than on  $\pi\pi^*$  state in gas phase. In contrast, the populations are prefer on  $\pi\pi^*$  state in MeCN. So, the fluorescence of BenzU will be enhanced in higher polarity solvent. In protic solvent, the more hydrogen bonds are formed between BenzU and protic solvent, the higher  $\Delta E$  will obtain. And then, the larger  $\Delta E$  will obtain higher fluorescence.

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