

CHEMISTRY A European Journal



Accepted Article

Title: Amphiphilic Fluorescence Resonance Energy Transfer Dyes: Synthesis, Fluorescence and Aggregation Behaviors in Water

Authors: Shilei Dou, Ying Wang, and Xin Zhang

This manuscript has been accepted after peer review and appears as an Accepted Article online prior to editing, proofing, and formal publication of the final Version of Record (VoR). This work is currently citable by using the Digital Object Identifier (DOI) given below. The VoR will be published online in Early View as soon as possible and may be different to this Accepted Article as a result of editing. Readers should obtain the VoR from the journal website shown below when it is published to ensure accuracy of information. The authors are responsible for the content of this Accepted Article.

To be cited as: Chem. Eur. J. 10.1002/chem.202000107

Link to VoR: http://dx.doi.org/10.1002/chem.202000107

Supported by ACES



Amphiphilic Fluorescence Resonance Energy Transfer Dyes: Synthesis, Fluorescence and Aggregation Behaviors in Water

Shilei Dou, Ying Wang and Xin Zhang*

Abstract: Amphiphilic pyrene/perylene bischromophore dyes were synthesized from unsymmetrically substituted perylene bisimide dyes, which were obtained by three synthetic methods. The optical behaviors and aggregation behaviors of these functional dyes were studied by UV/vis absorption and fluorescence spectroscopy, dynamic light scattering and transmission electron microscopy. These dyes are highly fluorescent covering whole visible light region. A donor/acceptor dye displays intramolecular fluorescence resonance energy transfer with a high efficiency up to 96.4% from pyrene to peylene bisimide chromophores, which leads to a high fluorescence color sensitivity to environment polarity. Under 365 nm UV lamp, the light-emitting colors of donor/acceptor dye change from green to yellow with increasing solvent polarity, which demonstrates application potentials as a new class of fluorescence resonance energy transfer probes. The donor/acceptor dye in water was assembled into hollow vesicles with narrow size distribution. The bilayer structure of vesicular wall was directly observed by transmission electron microscopy. These vesicular aggregates in water are fluorescent at 650-850 nm within near-infrared region.

Introduction

Fluorescence (Förster) resonance energy transfer (FRET) is a phenomenon of radiationless energy transfer occurring between two chromophores, specifically, a donor chromophore transfer energy to an acceptor chromophore through nonradiative dipole-dipole coupling when the donor is photo-excited.^[1] Fluorescence resonance energy transfer is involved in photosynthesis system in nature.^[2] Recently, it has attracted much attention due to diverse applications such as artificial photosynthetic light harvesting systems, bioimaging, biosensors and fluorescence probes,[3-5] and white light-emitting materials.[6. ^{7a]} Generally, the energy transfer efficiency mainly depends on three factors:^[4b,c] 1) donor-to-acceptor distance, 2) the spectral overlap of the acceptor absorption and the donor emission spectra,^[7] and 3) the chromophore orientation of the donors and the acceptors. The distance dependence of the energy transfer efficiency has been frequently used in chemical, biological and biophysical fields as spectroscopic ruler^[4b, 8] to measure the biomacromolecular distance generally in the range from 1 to 10 nm. Encouraged by these unique emission characteristics, chemists have designed and synthesized various functional dye molecules, and prepared their supramolecular assemblies to construct fluorescence resonance energy transfer systems. Recently, Ajayaghosh and coworkers have systemically studied highly efficient energy transfer systems constructed from oligo

S. Dou, Y. Wang, Prof. Dr. X. Zhang School of Chemical Engineering and Technology, Collaborative Innovation Center of Chemical Science and Chemical Engineering (Tianjin) Tianjin University, Tianjin, 300072, (China) E-mail: xzhangchem@tju.edu.cn (p-phenylenevinylene) donors and acceptors connected by supramolecular weak H-bonds $^{[1a,\ 1b,\ 6,\ 9]}$ or covalent bonds. $^{[10]}$

Perylene bisimides dyes (PBIs), one class of rylene dyes based on naphthalene units, possess outstanding optical properties such as high extinction coefficients and absorption at light region, strong electron-accepting visible ability, fluorescence quantum yields close to unity, and excellent chemical, photo-, thermal- stability.[11] Owing to these outstanding optical properties, perylene bisimide dyes recently have been extensively studied and their applications have been expanded tremendously in the last 25 years from traditional industrial dyes^[12] into various new fields including optoelectronic and n-type semiconductor materials,^[13] photovoltaic devices^[11] and solar cells,^[14] light-emitting diodes,^[15] field effect transistors^[16] and near-infrared dyes.^[17] More recently, owing to the strong π - π stacking interactions, pervlene bisimide dyes have been regarded as versatile building blocks for various supramolecular assemblies and aggregates in organic solution,^[17a, 18] even in water.^[19]

In our previous work, we have reported on precise control of dye aggregate morphologies including micelles, rods and vesicles in water by tuning molecular curvatures.^[21] Subsequently, we further developed water-soluble, white-light emitting intermolecular energy transfer system, where bispyrene donors were encapsulated in acceptor vesicles, and energy transfer efficiency is up to 74%.^[7a] Towards the purpose of creating an intramolecular energy transfer system, here, we synthesized a new amphiphilic energy transfer perylene bisimide dye where the acceptor and donor are covalently linked together in a single molecule (Figure 1). The optical properties and aggregation behaviors of the new intramolecular energy transfer dye were investigated in water.

Results and Discussion





WILEY-VCH



Figure 2. Three synthetic routes towards unsymmetrical perylene bisimide 2.

Synthesis. In this work, we first synthesized unsymmetrically substituted perylene bisimide dye 2 as energy acceptor (Figures 1 and 2). Compared with symmetrically substituted dyes, unsymmetrically substituted perylene bisimide dyes not only require more complex reaction steps and purification processes but also have lower yield. We thus developed three synthetic routes for unsymmetrically substituted dyes (Figure 2). First synthetic route is based on one-pot reaction procedure. Two different substituent groups, i. e., 3, 4, 5-tris(3-(2-(2ethoxyethoxy)ethoxy)-1-propyl)-aniline and 6-amino-hexanol, are mixed with the perylene-3, 4, 9, 10-tetracarboxylic dianhydride in a pot to perform the substitution reaction.[21] Owing to the competition of symmetric substitution, the first synthetic method eventually produces considerable by-products, resulting in difficulty in purification and extremely low yield (2%). The second and third synthetic routes are based on two-step reaction procedure. 3, 4, 5-Tris(3-(2-(2-ethoxyethoxy)ethoxy)-1propyl)-aniline was reacted with perylene- 3, 4, 9, 10dianhydride to produce monoanhydride tetracarboxylic monoimide and then 6-aminohexanol was reacted with monoanhydride monoimide at another imide position. In the third synthetic route, the reaction order of 3, 4, 5-tris(3-(2-(2ethoxyethoxy)ethoxy)-1-propyl)-aniline and 6-aminohexanol is opposite with the second route. Higher reaction yields (29~42%) were obtained by the two-step reaction of the second and third synthetic routes.

For the most reported unsymmetrically substituted perylene bisimide dyes,^[11a] although the substituent groups at two imide positions are different, but they are based on the same class of amino substituent groups, i.e. both aromatic amino groups or both alkyl amino groups at two imide positions. In our unsymmetrically substituted synthesis, the aromatic amino group was attached to one imide side, and the alkyl amino group was attached to the other imide side. The aromatic and alkyl amino groups have extremely different reaction activity, thus, our unsymmetrical substitution is a synthetic challenge. By using the third route, we obtained the highest reaction yield (42%, Figure 2) for unsymmetrical dye **2**. The dye **3** consists of pyrene donor **1** and perylene bisimide acceptor **2**, which was synthesized by the esterification of energy donor 1-pyrenylbutyric acid **1** with the hydroxyl group of alkyl chain of acceptor **2** (Figure 3).



Figure 3. Synthetic route towards donor/acceptor dye 3.

Fluorescence resonance energy transfer. To investigate the energy transfer of these dyes, we performed UV/vis absorption and fluorescence spectroscopic measurements. The optical data including maximum UV/vis absorption and fluorescence emission wavelengths, extinction coefficients, fluorescence lifetimes and fluorescence quantum yields of these dyes are listed in Table 1. The dichloromethane solution of donor 1 is transparent. 2 and 3 are slightly pink in dichloromethane (Figure 4a inset). Donor 1 and acceptor 2 show characteristic absorption of pyrene and perylene bisimide chromophores with well-defined

WILEY-VCH

fine structures and maximum absorption wavelengths at 344 nm and 525 nm, respectively (Figure 4a). In addition, the absorption bands of **1** and **2** are well separated, therefore, **1** and **2** can be selectively excited. Donor/acceptor dye **3** shows two characteristic absorption bands at 345 nm and 525 nm, which are obviously arose from pyrene and perylene bisimide chromophores, respectively. At the same concentration of these dyes in solution, the absorption spectrum of **3** is consistent with the absorption spectra of donor **1** plus acceptor **2** (Figure 4a), suggesting that although pyrene and perylene chromophores are connected by covalent bonds with a close distance in **3**, they have no interaction in the ground state and still maintain their independent optical properties.



Figure 4. UV/vis absorption (a) and fluorescence emisson spectra (b) of energy donor 1, acceptor 2 and donor/acceptor 3 in dichloromethane. $\lambda_{ex} = 330$ nm. $c = 2 \times 10^{-6}$ M. Inset in (a): Photograph of dichloromethane solution of 1, 2 and 3 under normal light. Inset in (b): Photograph of dichloromethane solution of 1 and 3 under 365 nm UV lamp and CIE 1931 chromaticity diagram. These points indicated by circles are the fluorescence color coordinates for 1 (0.17, 0.04) and 3 (0.36, 0.56). c) and d) Excitation-dependent 3D fluorescence spectra of 3 in dichloromethane. [3]= 2×10^{-6} M.

All these dyes **1-3** are highly fluorescent covering whole visible light region. The fluorescence quantum yields for donor **1**, acceptor **2** and donor/acceptor **3** were measured to be 0.17, 0.54 and 0.18, respectively. Fluorescence lifetimes were measured to be 31.45 ns, 3.59 ns and 0.99 ns for donor **1**, acceptor **2** and donor/acceptor **3**, respectively (Table 1). Donor **1** shows blue-purple fluorescence of pyrene chromophore with well-defined fine structure at 376 nm and 397 nm in dichloromethane (Figure 4b). Under 365 nm UV lamp, **1** emits

blue-purple light in dichloromethane with a light-emiting color coordinate (0.17, 0.04) in CIE 1931 chromaticity diagram (Figure 4b inset). Acceptor 2 displays green-yellow fluorescence from perylene bisimide chromophore with well-defined fine structure at 534 nm and 577 nm in dichloromethane. When donor/acceptor 3 was excited at 330 nm where only pyrene donor was excited, the fluorescence of pyrene donor was almost not observed, and enhanced fluorescence of perylene bisimide acceptor was observed (Figure 4b). Under 365 nm UV lamp, 3 emits only acceptor green-yellow light in dichloromethane with a light-emiting color coordinate (0.36, 0.56) (Figure 4b inset). These results suggest the fluorescence energy transfer occurring from pyrene donor to perylene acceptor in 3.[22] To confirm the energy transfer, we performed the excitationdependent 3D fluorescence spectral measurements (Figure 4 c and d). When 3 was selectively excited at 450-530 nm for its pervlene acceptor, or at 310-360 nm for its pyrene donor, we observed the same fluorescence spectra from pervlene bisimide acceptor. These results further reveal high efficient energy transfer occurring from pyrene donor to perylene acceptor in dye 3.

Table 1. Optical data of energy donor **1**, acceptor **2** and donor/acceptor **3** in CH₂Cl₂ ($c = 2 \times 10^{-6}$ M): maximum absorption wavelength (λ_A), maximum fluorescence emission wavelength (λ_F), extinction coefficient (ϵ), fluorescence lifetime (τ) and fluorescence quantum yield (ϕ).

| Dyes | λ _A /nm | ε /10 ⁴ M ⁻¹ cm ⁻¹ | λ _F /nm | τ /ns | ф | _ |
|------|--------------------|---|--------------------|-------|------|---|
| 1 | 344 | 5.44 | 376 | 31.45 | 0.17 | - |
| 2 | 525 | 7.19 | 534 | 3.59 | 0.54 | |
| 3 | 345, 525 | 3.53, 7.32 | 533 | 0.99 | 0.18 | |

To quantify the energy transfer of **3**, the efficiency (*E*) of energy transfer was calculated by the following equations:^[4c]

$$J(\lambda) = \frac{\int_0^\infty F_D(\lambda)\varepsilon_A(\lambda)\lambda^4 d\lambda}{\int_0^\infty F_D(\lambda)d\lambda}$$
(1)
$$R_0 = 0.211 [k^2 \eta^{-4} \phi_D J(\lambda)]^{\frac{1}{6}}$$
(2)

$$E = \frac{R_0^6}{R_0^6 + r_{DA}^6}$$
(3)

where λ is the wavelength, F_D (λ) is donor's fluorescence spectrum, ε_A is acceptor's extinction coefficient, J (λ) is the overlap integral of acceptor absorption and donor emission, k^2 is the orientation factor and usually takes the value of 2/3.^[23], η is the refractive index of the medium, ϕ_D is the fluorescence quantum yield of donors without acceptors, R_0 is the Förster distance, that is, the distance between donor and acceptor when the efficiency of energy transfer is 50%. r_{DA} is the donor-toacceptor distance. The energy-minimized molecular structure of **3** (semi-empirical AM1 calculations) indicates that donor-toacceptor distance (r_{DA}) is 14.6 Å (Supporting information S11). $J(\lambda)$ was calculated to be 1.087×10^{14} cm⁻¹M⁻¹m⁻⁴ according to the equation (1) and R_0 was then calculated to be 25.3 Å according to the equation (2). We finally obtained the energy transfer efficiency (*E*) as high as 96.4% according to the

WILEY-VCH

FULL PAPER

equation (3). This is a rare example for such high intramolecular efficient energy transfer. Energy transfer efficiency (*E*) could also be calculated on the basis of fluorescence lifetimes by equation:^[4c] $E = 1 - \tau_{DA}/\tau_D$, where τ_{DA} is 0.99 ns and τ_D is 31.45 ns, which are fluorescence lifetimes of donor/acceptor **3** and donor **1**, respectively. Energy transfer efficiency (*E*) was obtained to be 96.8% from fluorescence lifetimes, which well agrees with our above result (96.4%) obtained from the equations (1), (2) and (3).

Fluorescence resonance energy transfer probe for environmental polarity sensitivity. Donor /acceptor 3 is a new dye molecule. We further explore its application potentials as a new class of fluorescence resonance energy transfer probes. Generally, the emission wavelength of a fluorophore is longer than the absorption wavelength. This difference is known as Stokes' shift. The emission energy lever of a dye fluorophore is lower than its absorption energy lever. This energy loss is caused by several dynamic processes occurring during light absorption, such as vibration relaxation, the changes of dipole moments of excited state fluorophores, reorientation or relaxation of the solvent dipoles after excitation.^[4c, 24]



Figure 5. UV/vis absorption (a) and fluorescence emission spectra (b) of donor/acceptor **3** in cyclohexane (CYH), chloroform (CHCl₃), ethyl acetate (EtOAc), dichloromethane (DCM), tetrahydrofuran (THF), n-butanol (n-BuOH) and methanol (MeOH). **[3]** = 2×10⁻⁶ M, λ_{ex} = 344nm. c) Lippert plot for **3** in various solvents. d) Photograph of **3** in various solvents under 365 nm UV lamp. e) CIE 1931 chromaticity diagram. These points indicated by circles are the fluorescence color coordinates for **3** in ethyl acetate (0.39, 0.57), tetrahydrofuran (0.41, 0.55), dichloromethane (0.42, 0.54), n-butanol (0.42, 0.53).

To study the environment polarity sensitivity of **3**, we chosen the different polar solvents: cyclohexane (CYH), chloroform (CHCl₃), ethyl acetate (EtOAc), dichloromethane (DCM), tetrahydrofuran (THF), n-butanol (n-BuOH) and methanol (MeOH). UV/vis absorption and fluorescence emission spectra of **3** were measured at a concentration of 2×10^{-6} M (Figure 5a, b). Fluorescence behaviors of **3** display obviously

changes in different polar solvents (Figure 5b, Table 2). Under 365nm UV lamp, light-emitting colors of **3** change from green to yellow with increasing solvent polarity (Figure 5d). The light-emitting color coordinates in CIE 1931 chromaticity diagram move from (0.39, 0.57) in green region for **3** in ethyl acetate, to (0.42, 0.53) in yellow region for **3** in polar n-butanol (Figure 5e). We further used the empirical Lippert equations (4) and $(5)^{[4c]}$ to quantify the environment solvent polarity sensitivity:

$$v_A - v_F = \frac{2}{hc} \Delta f \frac{(\mu_E - \mu_G)^2}{a^3} + constant$$
(4)

$$\Lambda f = \frac{\varepsilon - 1}{2\varepsilon + 1} - \frac{n^2 - 1}{2n^2 + 1}$$
(5)

Where $\nu_{\mathcal{F}}$ and ν_{A} are the wavenumbers (cm⁻¹) of the emission and absorption, respectively, μ_{G} and μ_{E} are the dipole moments of dye **3** in the ground state and the excited state, respectively, *c* is the light speed, *h* is Planck's constant, *a* is the molecular radius of dye **3**, *n* and *c* are the refractive index and the dielectric constant of the solvents, respectively, and Δf is the orientation polarizability.

Table 2. Solvent environment polarity sensitivity. Stokes' shifts (v_{A-VF} or λ_{F} - λ_{A}) of **3** in various polar solvents. **[3]** = 2×10^{-6} M. Δf is solvent parameter (orientation polarizability),

| Solvents | Δf^a | λ _A /nm | λ _F /nm | va - v _F /cm ⁻¹ | $\Delta \lambda = \lambda_F - \lambda_A$ /nm |
|-----------------|--------------|-----------------------|-----------------------|--|---|
| Cyclohexane | -0.0026 | 519 | 521 | 74 | 2 |
| Chloroform | 0.1523 | 527 | 534 | 248 | 7 |
| Ethyl acetate | 0.1997 | 519 | 526 | 256 | 7 |
| THF | 0.2103 | 521 | 529 | 290 | 8 |
| Dichloromethane | 0.2182 | 525 | 533 | 285 | 8 |
| n-Butanol | 0.2644 | 527 | 536 | 318 | 9 |
| Methanol | 0.3086 | 528 | 538 | 352 | 10 |

^a $\Delta f = [(\epsilon - 1)/(2\epsilon + 1)] - [(n^2 - 1)/(2n^2 + 1)]$

Stokes' shifts of dye 3 show significantly increase with increasing the solvent orientation polarizability (Δf), revealing that 3 is very sensitive to polarity of the solvents. We plotted the Stokes' shifts ($v_A - v_F$) versus solvent polarity parameters (Δf) and a linear relationship was obtained (Figure 5c). According to the Lippert equations, the value of $(2/hc)[(\mu_E - \mu_G)^2/a^3]$ was obtained from the slope of the Lippert plot. The value of a is 22.7 Å. The change ($\Delta\mu$ = μ_E - μ_G) in dipole moment between the excited state and the ground state was thus calculated to be 32.1 D, which is comparable to the dipole moment change from one unit charge's (4.8×10⁻¹⁰ esu) separation by 6.7 Å. These results reveal that the excited state dipole moment of 3 changes significantly with the polarity of the solvents, which is due to the existence of fluorescence resonance energy transfer in 3 from pyrene to perylene chromophores. From all these results, we conclude that dye 3 is sensitivity to environment polarity, which can be regarded as a new class of fluorescence resonance energy transfer probes.

WILEY-VCH

FULL PAPER

Supramolecular assembly and aggregation behaviors in water towards nanosystem fluorescence sensors. We further explore the application of our novel fluorescence probe in biologically relevant environment, i.e. water. Before the study on aggregation in water, we first investigated the supramolecular assembly behaviors of 3 in organic solvents. Concentration-dependent UV/vis absorption spectra were measured in tetrahydrofuran (Figure 6). At low concentration (<10⁻⁶M), 3 displays the absorption spectrum of molecularly dissolved state. With increasing the concentration, the absorption spectra become broad, the extinction coefficient decreases, and the maximum absorption wavelength is blueshifted about 33 nm. At the same time, a new weak absorption band appears at about 553 nm, and an isosbestic point was observed at 537 nm (Figure 6). These absorption spectral changes reveal molecular aggregation occurring with increasing concentration in tetrahydrofuran through π - π stacking between the aromatic chromophores.



Figure 6. UV/vis absorption spectra of 3 in tetrahydrofuran at different concentration from 1×10⁻⁶ M to 1×10⁻² M. The arrows indicate changes of the spectra with increasing concentration. Inset: plot of mole fraction of aggregated molecules (α_{agg}) versus concentration in tetrahydrofuran. The curve was acquired by fitting the data according to the isodesmic model.^[25, 27]

The mole fraction of aggregated molecules (α_{agg}) is obtained by the following equation (6): $^{[26]}$

$$\alpha_{agg} = \frac{\varepsilon - \varepsilon_{mon}}{\varepsilon_{agg} - \varepsilon_{mon}} \tag{6}$$

where ε is the extinction coefficient and subscripts *mon* and *agg* are monomers and aggregates in solution respectively. It is assumed here that the molecules are fully aggregated at the highest concentration and all dissolved in molecular state at the lowest concentration. According to equation (6), the plot of the mole fraction of aggregated molecules (α_{agg}) versus the concentration in solution, reveals the changes from molecularly dissolved state to aggregated state in tetrahydrofuran. We subsequently measured concentration-dependent fluorescence spectroscopy (Figure S13). A new structureless and broad fluorescence band at long wavelength appears and increases gradually with increasing the concentration, which further confirms the aggregate formation in tetrahydrofuran.

Donor/acceptor 3 is an amphiphilic dye composed of three hydrophilic oligo-ethylene glycol chains and hydrophobic pyrene/perylene bischromophores. We further investigated its aggregation behaviors in water by UV/vis absorption (Figure 7a) and fluorescence emission spectroscopy (Figure 7b, c). Tetrahydrofuran is a good solvent for 3, thus, 3 dissolves in tetrahydrofuran (THF) in molecularly dissolved state at low concentration. Water is a poor solvent. As the fraction of water in tetrahydrofuran solution increases, the absorption maximum wavelength was blue-shifted from 525 nm to 500 nm. Simultaneously, a decrease in the extinction coefficient and a loss of the spectral fine structures were observed (Figure 7a). Moreover, a new broad absorption band appears at a longer wavelength of around 550 nm. When water fraction is larger than 50% (>50%, v/v), UV/vis absorption spectroscopic baseline of 3 does not go to zero, indicating the suspension characteristics of the aggregate formation.



Figure 7. UV/Vis absorption (a) and fluorescence emission spectra (b) of **3** in THF/H₂O. λ_{ex} = 344nm. [**3**] = 2×10⁻⁶ M. The arrows indicate the spectral changes upon increasing the fraction of water. Inset in (b): CIE 1931 chromaticity diagram. These points indicated by circles are the fluorescence color coordinates for **3** in THF/H₂O: 100/0 (0.36, 0.61), 80/20 (0.37, 0.59), 50/50 (0.38, 0.56), 40/60 (0.36, 0.52), 35/65 (0.27, 0.30), 10/90 (0.20, 0.11). c) Fluorescence spectra of **3** in THF/H₂O. [**3**] = 2×10⁻⁶ M. λ_{ex} =344nm. d) Photograph of **3** in THF/H₂O under 365 nm UV lamp. [**3**] = 2×10⁻⁶ M.

As the solvents change from nonpolar THF to polar water, the fluorescence intensity of dye **3** gradually decreases (Figure 7 b), a new fluorescence band appears, and the maximum emission wavelength at 529 nm is red-shifted to 755 nm, which falls within near infrared region (Figure 7c). The large red-shift (226 nm) of emission wavelength with increasing solvent polarity further confirms our above conclusion, that is, environment polarity sensitivity of dye **3**. The new broad near-infrared fluorescence band becomes more obvious at a higher concentration in water (Figure S14). Figure 7d shows a photograph of **3** from molecularly dissolved state to aggregating state in THF/water under 365nm UV lamp. Fluorescence color coordinates of **3** in CIE 1931 chromaticity diagram in THF/water are shown in Figure 7b inset, which indicates the color changes

WILEY-VCH

FULL PAPER

with increasing the water fraction. All these spectral results reveal the large aggregate formation of **3** in aqueous solution.

We subsequently performed theoretical analysis by using Israelachvili's models for aggregates of 3 in water.^[21, 28] These theoretical models are based on molecular curvatures to produce a minimum in free energy for assembling morphology in polar solvents by using Israelachvili's critical packing parameter $P_c = V/aI$, where V and I are the effective volume and optimal length of molecule, respectively, a is the area occupied by hydrophilic chains. This Israelachvili's model is particularly suitable to predict the assembly of shape-preserving robust molecules, where no change in molecular length and the hydrophilic occupied area. According to Israelachvili's models, with increasing critical packing parameter P_c , the spontaneous curvature increases on the surface between aqueous and organic phases, and assembling morphologies change from spherical micelles ($P_c < 0.33$), cylindrical or disk-shaped micelles $(0.33 \le P_c \le 0.5)$, vesicular structures $(0.5 \le P_c \le 1.0)$, to inverted or reversed micelles ($P_c > 1.0$). For dye 3, critical packing parameter (P_c) was calculated to be 0.61, which theoretically predicts that vesicular aggregates should be favorably formed in polar solvents.



Figure 8. a) TEM images of aggregates of **3** prepared from deionized water. Top and inset: magnified TEM images of one typical bilayer vesicle. The outer and inner layers are composed of approximate 4.1×10^5 and 3.5×10^5 molecules, respectively. b) Size distribution of the aggregates calculated from 99 aggregates according to TEM measurements. c) Size distribution of aggregates of **3** obtained from dynamic light scattering in water. d) Photograph of aggregates of **3** in water under 365 nm UV lamp. e) Excitation-dependent 3D fluorescence spectra of aggregates of **3** in deionied water. [**3**] = 0.12 mg/ml.

The aggregate morphologies of 3 in water were then observed by transmission electron microscopy (TEM). The TEM sample of 3 was prepared as follows: 3 (0.36 mg) was dissolved in THF (6 mL), and then the deionized water (3 mL) was slowly added into the above solution. The suspension solution was exposed to air at room temperature for several days to remove tetrahydrofuran by volatilization. The suspension solution of 3 in water was finally obtained at a concentration of 0.12 mg/ml, which was then used for TEM measurement. Our theoretical prediction was confirmed by TEM observation, which reveals the formation of hollow vesicles with an average size of about 62 nm in water (Figure 8a-b). The wall thickness is uniform (9-10 nm), which is corresponding to twice the length of one molecule (5.3 nm in the extended conformation of alkyl spacer, 4.5 nm in the coiled conformation of alkyl spacer). Interestingly, the bilayer structures were directly observed by TEM (Figure 8a inset). The thickness of outer and inner layer is 5-6 nm and 4-5 nm, respectively, implying the extended alkyl spacer for outer layer molecules, and the coiled alkyl spacer for inner layer molecules. To the best of our knowledge, this is the first time to clearly and directly observe bilayer structures of vesicle by TEM. By dividing the volume of the double layer by the volume of a single molecule, the aggregate numbers of the outer and inner layers were calculated to be approximate 4.1×10^5 and 3.5×10^5 , respectively. These vesicles are well separated and isolated, close but not connected together, implying that these vesicles are not adhesive, and the bilayer membrane is rigid and no molecular exchanges occur intermembrane.

The above TEM results were further confirmed by dynamic light scattering (DLS) measurements. We observed a narrow scattering intensity distribution for aggregates of 3 in water (Figure 8c), suggesting the size monodispersity and structural homogeneity of these aggregates. DLS analysis indicates the average diameter of these vesicular aggregates is 149 nm in deionized water. DLS result is slightly larger than that from TEM, which can be attributed to the measured states of samples.^[29] In the case of DLS measurements, the samples were detected in situ in water. In the case of TEM measurements, the samples were observed in the dried state in vacuum chamber. Then we performed the excitation-dependent 3D fluorescence spectral measurement (Figure 8e), which indicates that these vesicular aggregates are light-emitting from 630 nm to 850 nm in nearinfrared region. Under 365 nm UV lamp, aggregates of 3 in water emits slightly dark red light (Figure 8d), which is not easily observed by naked eyes, because the light-emitting color beyond the visible light region, which falls within near-infrared region.

Conclusion

designed and synthesized We new pyrene/perylene bischromophore dyes from unsymmetrically substituted perylene bisimide dyes by three synthetic routes. The new dye demonstrates a high efficient fluorescence resonance energy transfer from pyrene donor to perylene bisimide acceptor. Owing to energy transfer effect, the fluorescence behaviors of the donor/acceptor dye are sensitive to polarity changes of microenvironment, which demonstrates application potentials as novel fluorescence probe. The donor/acceptor dye assembled into bilayer vesicles in water with narrow size distribution revealed by UV/vis absorption and fluorescence spectroscopy,

WILEY-VCH

and transmission electron microscopy and dynamic light scattering measurements. Our results may encourage the effort to develop new class of fluorescence probes and functional dye aggregates in water.

Experimental Section

Dye 2: 3, 4, 5-tris(3-(2-(2-ethoxyethoxy)ethoxy)-1-propyl)-aniline (0.22 g, 0.45 mmol), monoanhydride monoimide (0.13 g, 0.23 mmol) and zinc acetate (0.04 g, 0.22 mmol) were mixed with imidazole (3.47 g, 0.05 mol). The reaction mixture was heated under nitrogen at 140°C. After 12 hr. the reaction mixture was cooled to room temperature. 1M HCl (75 mL) was added to the above reaction mixture. The resulting mixture was extracted with dichloromethane (75 mL) for several times. Then the solution was dried with anhydrous MgSO4 and concentrated under reduced pressure, the crude product was purified by silica gel column chromatography using dichloromethane/methanol (40/1, v/v) as eluent to give a red solid (150mg, 60%). Rf = 0.31 (CH₂Cl₂/CH₃OH=20/1, v/v). ¹H NMR (400MHz, CDCl₃, ppm)(Figure S5): δ= 8.56-8.72 (m, 8H, ArH in perylene ring), 7.04 (s, 2H, ArH in phenyl ring), 4.21 (t, J=7.6 Hz, 2H, -NCH2-), 3.73-3.54 (m, 32H, 15-OCH2-, -CH2OH), 3.51 (q, J=2.8 Hz, $\mathsf{J=17.2}\ \mathsf{Hz},\ \mathsf{6H},\ \mathsf{3\text{-}OCH_2CH_3}),\ \mathsf{2.78}\ (\mathsf{t},\ \mathsf{J=8.00}\ \mathsf{Hz},\ \mathsf{6H},\ \mathsf{3\text{-}PhCH_2\text{-}}),\ \mathsf{2.00\text{-}}$ 1.93 (m, 4H, 2-PhCH₂CH₂-), 1.91- 1.77(m, 4H, -PhCH₂CH₂-, -NCH₂CH₂), 1.54-1.46(m, 4H, -CH₂CH₂CH₂OH) 1.23 (t, J= 7.2 Hz, 6H, 2-CH₃), 1.17 (t, J= 6.8 Hz, 3H, -CH₃). MALDI-TOF MS (matrix: α-cyano-4hydroxycinnamic acid) calculated for C83H94N2O15 1088.561 m/z, found 1111.737 [M+Na]+.

Dye 3: 1-Pyrenebutyric acid 1 (50 mg, 0.17 mmol), 2 (169 mg, 0.15 mmol). 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC·HCI) (150 mg, 0.79 mmol) and 4-dimethylaminopyridine (DMAP) (10 mg, 0.08 mmol) were dissolved in dichloromethane (10 mL). The reaction mixture was stirred at room temperature. After 12 hr, the reaction mixture was concentrated under reduced pressure. Then the residue was extracted with water/dichloromethane. The resulting dichloromethane solution was dried with anhydrous MgSO4 and concentrated under reduced pressure, the crude product was purified by silica gel column chromatography using dichloromethane/methanol (70/1) as eluent to give a red solid (163 mg, 80%). Rf = 0.26 (CH₂Cl₂/CH₃OH = 40/1). ¹H-NMR (400MHz, CDCl₃, ppm)(Figure S6): δ= 8.56-8.45 (dd, J=7.6 Hz, J=37.2 Hz, 4H, ArH in perylene ring), 8.29-8.36 (dd, J=3.6 Hz, J=20.4 Hz, 4H, ArH in perylene ring), 7.69-8.10(m, 9H, ArH in pyrene ring), 7.05 (s, 2H, ArH in phenyl ring), 4.18 (m, 4H, -N-(CH₂)₅CH₂-), 4.15 (t, J=6 Hz, 2H, -NCH2-), 3.75-3.44 (m, 36H, 18-OCH2-), 3.16 (t, J=7.6 Hz, 2H, Ar(perylene ring)CH₂-), 2.80 (m, 6H, 3ArCH₂-), 2.43 (t, 2H, J=6.8 Hz, Ar(perylene ring)-CH2CH2CH2), 2.13-2.01(m, 2H, -NCH2CH2), 2.01-1.91(m, 4H, -PhCH₂CH₂-), 1.91-1.77(m, 4H, Ar(perylene ring)-CH₂CH₂-, PhCH₂CH₂-), 1.77-1.69(m, 2H, -NCH₂CH₂CH₂-), 1.56-1.47(m, 4H, N(CH₂)₃CH₂CH₂), 1.23 (t, J=6.8 Hz, 3H, -CH₃), 1.17 (t, J= 6.8 Hz, 6H, 2-CH₃). ¹³C-NMR (CDCl₃, ppm) (Figure S7): δ= 173.45, 163.38, 163.11, 141.50, 141.19, 138.83, 135.71, 134.21, 134.10, 133.86, 132.60, 131.09, 131.04, 130.74, 130.56, 129.60, 129.14, 128,62, 128.36, 127.24, 127.09, 127.04, 126.83, 126.39, 125.63, 124.68, 124.64, 124.57, 124.52, 123.16, 123.09, 122.76, 122.71, 122.60, 122.56, 71.26, 70.81, 70.71, 70.65, 70.56, 70.32, 70.14, 70.08, 69.91, 69.84, 68.47, 66.67, 66.60, 64.33, 40.33, 33.96, 32.60, 30.92, 29.99, 29.30, 28.32, 27.98, 26.74, 26.42, 25.66, 15.20, 15.15. MALDI-TOF MS (matrix: α-cyano-4-hydroxycinnamic acid) (Figure S9) calculated for $C_{83}H_{94}N_2O_{15},\,1358.67m/z,\,found\,\,1381.68$ [M+Na]⁺, 1397.69[M+K]⁺. IR (KBr pellet, cm⁻¹) (Figure S10): 2953.72 (VCH3), 2920.78 (Vanti,CH2), 2851.71 (Vsys,CH2), 1730.76, 1694.48, 1655.29, 1593.30, 1577.87, 1460.39, 1436.93, 1402.38, 1356.30, 1345.55, 1254.06, 1106.18, 1023.62, 950.53, 847.19, 808.61, 743.90.

Acknowledgements

We acknowledge the National Natural Science Foundation of China (Grant No. 21674079 and Grant No. 21975177) for financial support.

Conflict of interest

The authors declare no conflict of interest.

Keywords: Fluorescence • Fluorescence resonance energy transfer (FRET) • Perylene bisimides • Supramolecular assembly Aggregates • Dyes

- a) A. Ajayaghosh, V.K. Praveen, C. Vijayakumar, S. J. George, *Angew. Chem., Int. Ed.* 2007, *46*, 6260-6265. b) A. Ajayaghosh, V. K. Praveen, C. Vijayakumar, *Chem. Soc. Rev.* 2008, *37*, 109-122. c) P. Rajdev, S. Ghosh, *J. Phys. Chem. B* 2019, *123*, 327-342.
- a) D. Spitzer and P. Besenius, *Supramolecular Chemistry in Water*, Vol. 8 (Eds.: S. Kubik), VCH, Weinheim **2019**, pp. 285-336. b) T. Polivka, H. A. Frank, *Acc. Chem. Res.* **2010**, *43*, 1125-1134.
- a) J. Y. Shi, C. Y. Chan, Y. T. Pang, W. W. Ye, F. Tian, J. Lyu, Y. Zhang, M. Yang, *Biosens. Bioelectron.* **2015**, 67, 595-600; b) X. L. Zhang, Y. Xiao, X. H. Qian, *Angew. Chem., Int. Ed.* **2008**, 47, 8025-8029; c) L. Yuan, W. Y. Lin, K. B. Zheng, S. S. Zhu, *Acc. Chem. Res.* **2013**, 46, 1462-1473.
- a) B. Schuler, W. A. Eaton, *Curr. Opin. Struct. Biol.* 2008, *18*, 16-26; b)
 B. Prevo, E. J. G. Peterman, *Chem. Soc. Rev.* 2014, *43*, 1144-1155.c)
 J. R. Lakowicz, *Principles of Fluorescence Spectroscopy*, Springer, New York, 2006, pp. 205-472.
- J. Tang, B. Kong, H. Wu, M. Xu, Y. C. Wang, Y. L. Wang, D. Y. Zhao, G. F. Zheng, *Adv. Mater.* 2013, *25*, 6569-6574.
- [6] C. Vijayakumar, V. K. Praveen, A Ajayaghosh, Adv. Mater. 2009, 21, 2059-2063.
- [7] a) X. Zhang, S. Rehm, M. M. Safont-Sempere, F. Wurthner, *Nat. Chem.* 2009, *1*, 623-629; b) F. Li, X. H. Li, Y. Wang, X. Zhang. *Angew Chem Int Ed* 2019, *58*, 17994-18002; c) I. Z. Steinberg, *Annu. Rev. Biochem.* 1971, *40*, 83-114; d) R. P. Haugland, J. Yguerabide, L. Stryer, *Proc. Natl. Acad. Sci. U. S. A.* 1969, *63*, 23-30.
- [8] I. H. Stein, V. Schuller, P. Bohm, P. Tinnefeld, T. Liedl, ChemPhysChem 2011, 12, 689-695.
- a) S. S. Babu, K. K. Kartha, A. Ajayaghosh, *J. Phys. Chem. Lett.* 2010, 1, 3413-3424; b) V. K. Praveen, S. J. George, R. Varghese, C. Vijayakumar, A. Ajayaghosh, *J. Am. Chem. Soc.* 2006, *128*, 7542-7550; c) A. Ajayaghosh, C. Vijayakumar, V. K. Praveen, S. S. Babu, R. Varghese, *J. Am. Chem. Soc.* 2006, *128*, 7174-7175.
- [10] a) G. J. Qi, L. L. Jiang, Y. Y. Zhao, Y. Q. Yang, X. Y. Li, *Phys. Chem. Chem. Phys.* **2013**, *15*, 17342-17353; b) A. Sautter, B. K. Kaletas, D. G. Schmid, R. Dobrawa, M. Zimine, G. Jung, I. H. M. van Stokkum, L. De Cola, R. M. Williams, F. Wurthner, *J. Am. Chem. Soc.* **2005**, *127*, 6719-6729.
- a) Z. Chen, V.Stepanenko, V. Dehm ,P.Prins, L. D. A. Siebbeles, J. Seibt, P.Marquetand, V. Engel, F. Würthner, *Chem. Eur. J.* 2007, *13*, 436-449. b) C. Li, H. Wonneberger, *Adv. Mater.* 2012, *24*, 613-636.
- [12] W. Herbst, K. Hunger, Industrial Organic Pigments, VCH, Weinheim, 2004, 474-482.
- [13] a) K. Y. Chen, T. J. Chow, *Tetrahedron Lett.* 2010, *51*, 5959-5963; b) R.
 K. Gupta, D. S. S. Rao, S. K. Prasad, A. S. Achalkumar, *Chem. Eur. J.* 2018, *24*, 3566-3575.
- [14] a) J. L. Li, F. Dierschke, J. S. Wu, A. C. Grimsdale, K. Mullen, *J. Mater. Chem.* 2006, *16*, 96-100; b) X. D. Jiang, Y. H. Xu, X. H. Wang, F. Yang, A. D. Zhang, C. Li, W. Ma, W. W. Li, *Polym. Chem.* 2017, *8*, 3300-3306; c) D. Basak, D. S. Pal, T. Sakurai, S. Yoneda, S. Seki, S. Ghosh, *Phys. Chem. Chem. Phys.* 2017, *19*, 31024-31029; d) E. Kozma, M. Catellani, *Dyes Pigm.* 2013, *98*, 160-179.
- [15] a) R. K. Gupta, D. S. Shankar Rao, S. K. Prasad, A. S. Achalkumar, *Chem. Eur. J.* **2018**, *24*, 3566–3575. b) Y. Wang, Q. Zhang, F. Li, J. Gong, X. Zhang, *Dyes Pigm.* **2020**, *172*, 107823. c) M. Matussek, M. Filapek, P. Gancarz, S. Krompiec, J. G.Małecki, S. Kotowicz, M. Siwy,

WILEY-VCH

S. Maćkowski, A. Chrobok, E.S. Balcerzak, A. Słodek, *Dyes Pigm.* 2018, *159*, 590. d) Guner, T.; Aksoy, E.; Demir, M. M.; Varlikli, C. *Dyes Pigm.* 2019, *160*, 501–508. e) M. J. Taublaender, F. Glçcklhofer, M. Mar-chetti-Deschmann, M. M. Unterlass, *Angew. Chem. Int. Ed.* 2018, *57*, 12270 -12274; *Angew. Chem.* 2018, *130*, 12450 -12454. f) L. Yang, W. Gu, L. Lv, Y. Chen, Y. Yang, P. Ye, J. Wu,L. Hong, A. Peng and H. Huang, *Angew. Chem. Int.* 2018, *57*, 1096–1102.

- a) L. Schmidt-Mende, A. Fechtenkotter, K. Mullen, E. Moons, R. H. Friend, J. D. MacKenzie, *Science* 2001, 293, 1119-1122; b) C. Huang, W. J. Potscavage, S. P. Tiwari, S. Sutcu, S. Barlow, B. Kippelen, S. R. Marder, *Polym Chem.* 2012, *3*, 2996-3006.
- [17] a) S. Yagai, M. Usui, T. Seki, H. Murayama, Y. Kikkawa, S. Uemura, T. Karatsu, A. Kitamura, A. Asano, S. Seki, *J. Am. Chem. Soc.* 2012, *134*, 7983-7994; b) Q. L. Zhao, K. Li, S. J. Chen, A. J. Qin, D. Ding, S. Zhang, Y. Liu, B. Liu, J. Z. Sun, B. Tang, *J. Mater. Chem.* 2012, *22*, 15128-15135.
- [18] a) G. Golubkov, H. Weissman, E. Shirman, S. G. Wolf, I. Pinkas, B. Rybtchinski, *Angew. Chem., Int. Ed.* 2009, *48*, 926-930; b) H. Kar, S. Ghosh, Isr. *J. Chem.* 2019, *59*, 881-891; c) S. Ghosh, X. Q. Li, V.Stepanenko, F. Wurthner, *Chem. Eur. J.* 2008, *14*, 11343-11357.
- [19] a) R. Appel, J. Fuchs, S. M. Tyrrell, P. A. Korevaar, M. C. A. Stuart, I. K. Voets, M. Schonhoff, P. Besenius, *Chem. Eur. J.* **2015**, *21*, 19257-19264; b) M. M. Sun, K. Mullen, M. Z. Yin, *Chem. Soc. Rev.* **2016**, *45*, 1513-1528; c) K. Kondo, J. K. Klosterman, M. Yoshizawa, *Chem. Eur. J.* **2017**, *23*, 16710-16721.
- [20] T. M. Figueira-Duarte, K. Mullen, Chem. Rev. 2011, 111, 7260-7314.
- [21] a) X. Zhang, Z. J. Chen, F. Wurthner, J. Am. Chem. Soc. 2007, 129, 4886-4887. b) X. Zhang, D. Gorl, V. Stepanenko, F. Wurthner, Angew. Chem. Int. Ed. 2014, 53, 1270-1274; Angew. Chem. 2014, 126, 1294-1298.
- [22] D. Basak, A. Das, S. Ghosh, RSC Adv. 2014, 4, 43564-43571.
- [23] I. Z. Steinberg, J. Chem. Phys. 2003, 48, 2411-2413.
- [24] a) S. Sengupta, U. K. Pandey, *Org. Biomol. Chem.* 2018, *16*, 2033-2038; b) P. R. Aswathy, S. Sharma, N. P. Tripathi, S. Sengupta, *Chem. Eur. J.* 2019, *25*, 14870-14880.
- [25] a) J. Seibt, P. Marquetand, V. Engel, Z. Chen, V. Dehrn, F. Wurthner, *Chem. Phys.* **2006**, *328*, 354-362; b) N. J. Hestand, F. C. Spano, *Chem. Rev.* **2018**, *118*, 7069-7163.
- [26] L. Yang, G. Fan, X. Ren, L. Zhao, J. Wang, Z. Chen, *Phys. Chem. Chem. Phys.* 2015, 17, 9167-9172.
- [27] Martin, R. Bruce, Chem. Rev., 1996, 96, 3043-3064.
- [28] J. N. Israelachvili, Intermolecular and Surface Forces, Academic, San Diego, CA, 2011, pp.535-569.
- [29] W. C. She, K. Luo, C. Y. Zhang, G. Wang, Y. Y. Geng, L. Li, B. He, Z. W. Gu, *Biomaterials* **2013**, *34*, 1613-1623.

WILEY-VCH

Entry for the Table of Contents



An amphiphilic bischromophore dye was synthesized by three optimized synthetic routes. The new dye demonstrates high efficient intramolecular fluorescence resonance energy transfer from pyrene donor to perylene bisimide acceptor. Near-infrared fluorescent, narrow size distribution bilayer vesicles were prepared in water towards nanosystem fluorescence sensors.