Characterization of the Photophysical Behavior of DFHBI Derivatives: Fluorogenic Molecules that Illuminate the Spinach RNA Aptamer

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Determination of pK_a from the absorption spectra:

The pK_a of PFP-DFHBI can be determined from equation (1) in the main text, where we used absorbance data at 364 nm and 423 nm as a function of pH to obtain the ratio of concentraions of the anionic and neutral species:

$$A_n(\lambda_{364}) = \epsilon_n(\lambda_{364}) \cdot L \cdot [\text{neutral }]$$
$$A_a(\lambda_{423}) = \epsilon_a(\lambda_{423}) \cdot L \cdot [\text{anion}] \quad \text{(S1)}$$

$A_n(\lambda)$, $A_a(\lambda)$, $\epsilon_n(\lambda)$ and $\epsilon_A(\lambda)$ denote the absorbance of the neutral form, the absorbance of the anionic form, the molar absorptivity of the neutral form, and the molar absorptivity of the anionic form, respectively, at wavelength, λ. L is the path length of the cuvette. If all the fluorophores exist as the neutral form at the lowest pH (2.7) and as the anions at the highest pH (7.4), we can find the molar absorptivity ratio as:

$$\frac{\epsilon_n(\lambda_{364})}{\epsilon_a(\lambda_{423})} = \frac{A_n(\lambda_{364}, \text{pH} = 2.7)}{A_a(\lambda_{423}, \text{pH} = 7.4)} = \frac{A(\lambda_{364}, \text{pH} = 2.7)}{A(\lambda_{423}, \text{pH} = 7.4)} \quad \text{(S2)}$$

The net absorbances at 364 and 423 nm are given by:

$$A(\lambda_{364}) = A_n(\lambda_{364}) + A_a(\lambda_{364})$$
$$A(\lambda_{423}) = A_n(\lambda_{423}) + A_a(\lambda_{423}) \quad \text{(S3)}$$

Since the absorbance of the neutral form at 423 nm, $A_n(\lambda_{423})$, and the anionic form at 364 nm, $A_a(\lambda_{364})$, are nonzero they need to be subtracted from the absorbance at the corresponding wavelength. The contributions from the neutral form and the anion can be assumed to be the smallest at 423 nm and 364 nm, respectively, *i.e.* $A_n(\lambda_{423}) = A(\lambda_{423}, \text{pH} = 2.7)$ and $A_a(\lambda_{364}) = A(\lambda_{364}, \text{pH} = 7.4)$. Using these relations and equations (S1)-(S3), we obtain equation (2) in the main text.

Table S1

Fluorescence quantum yields for DFHBI and PFP-DFHBI

Fluorophores	ϕ_F (λ_{ex} = 460 nm)[a]
DFHBI	47.3
PFP-DFHBI	40.6

[a] Ratio of the fluorescence quantum yields, at the excitation wavelength of 460 nm, of the fluorophore in solid solution to that in liquid solution.

Figure S1

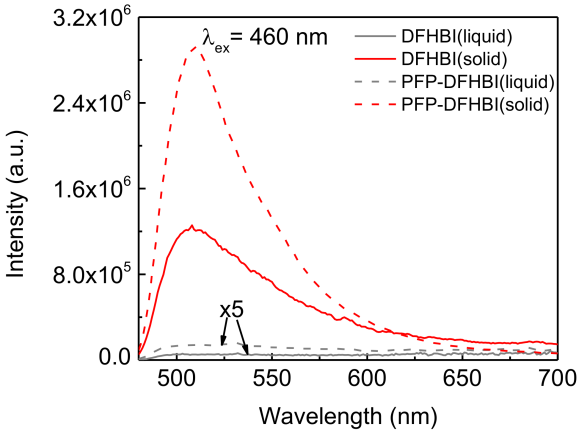


Figure S1. Fluorescence emission spectra of DFHBI (solid line) and PFP-DFHBI (dashed line) at $\lambda_{ex} = 460$ nm in liquid (gray) solvent (an equivolume mixture of methanol and ethanol) at room temperature and in solid (red) solvent at 77 K. The intensity scale of the spectra in liquid has been magnified 5 times.

Figure S2

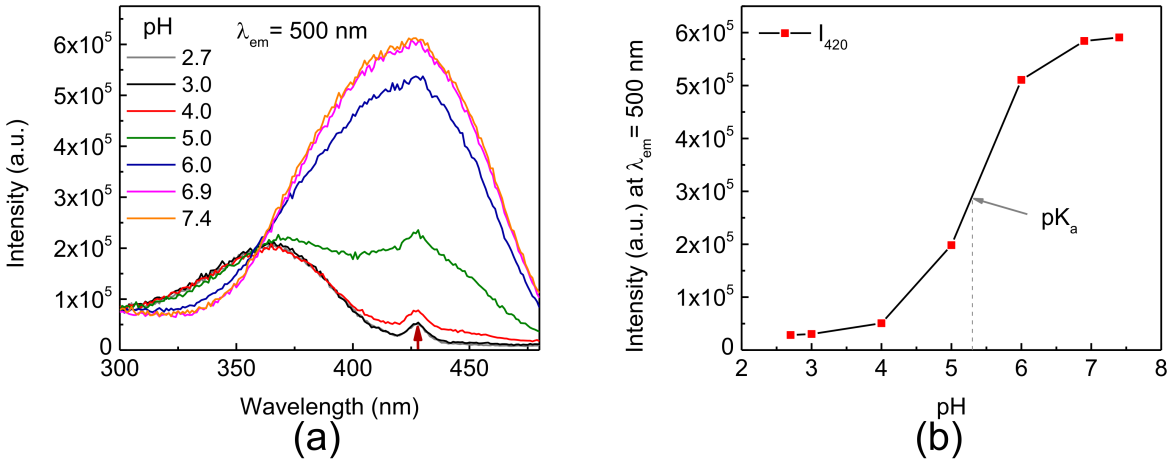
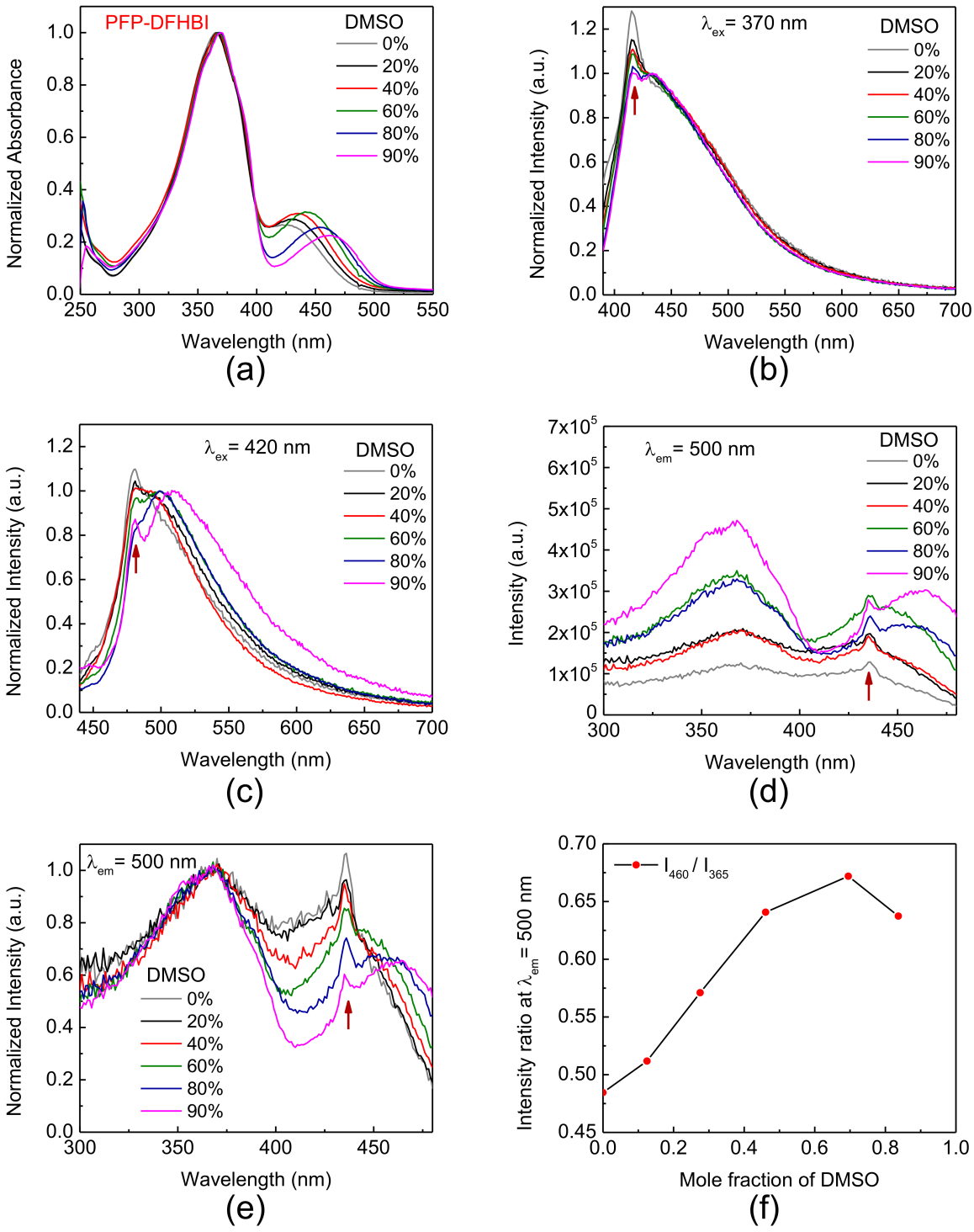


Figure S2. (a) Excitation spectra of PFP-DFHBI by collecting the fluorescence emission at $\lambda_{\mathrm{em}} =$ 500 nm. The band indicated by the dark red arrow is originated from the scattering from the solvents. (b) Emission intensity at $\lambda_{\mathrm{em}} = 500$ nm for the excitation at $\lambda_{\mathrm{ex}} = 420$ mm as a function of pH. The transition point where two species are present in an equal amount is indicated by the pKa value.

Figure S3


PFP-DFHBI
DMSO
0%
20%
40%
60%
80%
90%
Normalized Absorbance
Wavelength (nm)
(a)
λex= 370 nm
Normalized Intensity (a.u.)
(b)
λex= 420 nm
(c)
λem= 500 nm
Intensity (a.u.)
(d)
λem= 500 nm
(e)
I460 / I365
Intensity ratio at λem= 500 nm
Mole fraction of DMSO
(f)

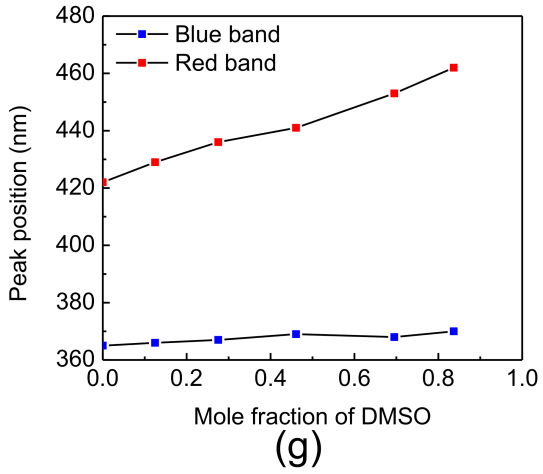
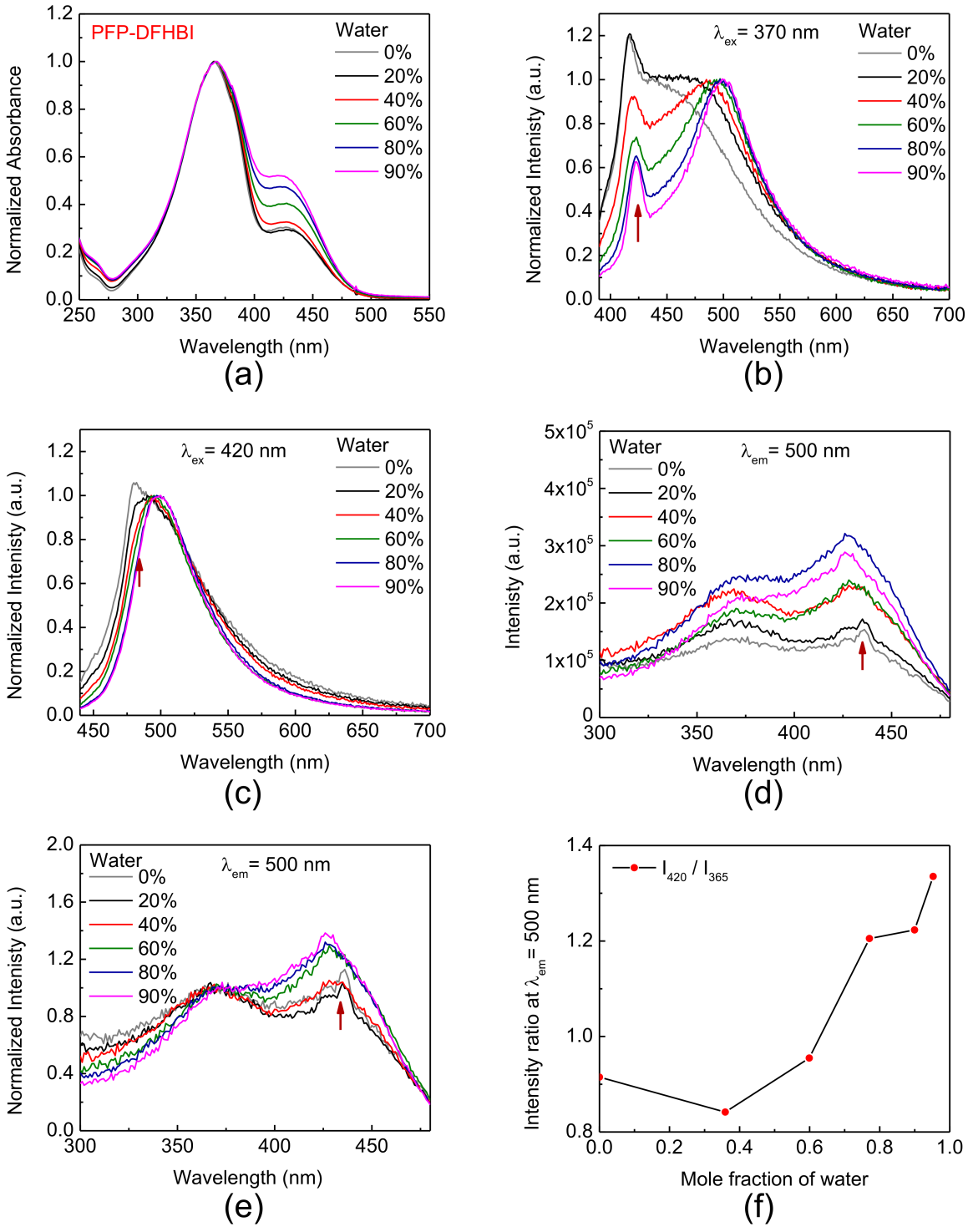


Figure S3. Spectral data of PFP-DFHBI in a series of DMSO-methanol mixtures. The percentages (v/v) of DMSO are indicated in the graphs. (a) Absorption spectra normalized at $\lambda_{\text{abs}}^{\text{max}}$(blue) given in **Table 2**. (b)-(c) Fluorescence emission spectra at $\lambda_{\text{ex}} = 370$ nm and $\lambda_{\text{ex}} = 420$ nm, respectively, normalized at corresponding $\lambda_{\text{em}}^{\text{max}}$ given in **Table 2.** (d) Excitation spectra obtained by collecting the fluorescence emission at $\lambda_{\text{em}} = 500$ nm. (e) Normalized excitation spectra, where normalization is carried out at the blue peak around 370 nm. In (b)-(e), the bands indicated by the dark red arrows arise from scattering from the solvent. (f) The ratio I_{460}/I_{365} as a function of the mole fraction of DMSO, where I_{460} and I_{365} are the emission intensities at 500 nm for the excitation at 460 and 365 nm respectively. This indicates that 460-nm excitation generates more 500-nm emission than does 365-nm excitation towards the higher percentage of DMSO. (g) The position of the peak maxima of the two absorption bands (the blue and the red for shorter and longer wavelengths respectively) as a function of the mole fraction of DMSO. This indicates that the red band, which corresponds to the ionized form of the fluorophore, shows a significant red shift.

Figure S4


PFP-DFHBI
Water
0%
20%
40%
60%
80%
90%
Normalized Absorbance
Wavelength (nm)
(a)
λex = 370 nm
Normalized Intenisty (a.u.)
(b)
λex = 420 nm
(c)
λem = 500 nm
Intenisty (a.u.)
(d)
(e)
I420 / I365
Intensity ratio at λem = 500 nm
Mole fraction of water
(f)

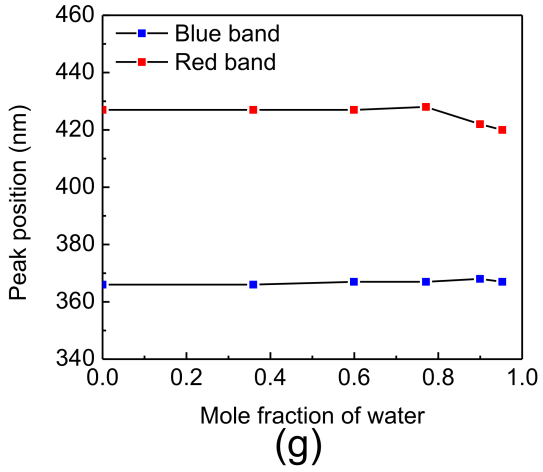


Figure S4. Spectral data of PFP-DFHBI in a series of water-methanol mixtures. The percentages (v/v) of water are indicated in the graphs. (a) Absorption spectra normalized at $\lambda_{\mathrm{abs}}^{\mathrm{max}}$(blue) given in **Table 3**. (b)-(c) Fluorescence emission spectra at $\lambda_{\mathrm{ex}} = 370$ nm and $\lambda_{\mathrm{ex}} = 420$ nm, respectively, normalized at corresponding $\lambda_{\mathrm{em}}^{\mathrm{max}}$ given in **Table 3.** (d) Excitation spectra obtained by collecting the fluorescence emission at $\lambda_{\mathrm{em}} = 500$ nm. (e) Normalized excitation spectra, where normalization is carried out at the blue peak around 365 nm. In (b)-(e), the bands indicated by the dark red arrows arise from scattering from the solvent. (f) The ratio I_{420}/I_{365} as a function of the mole fraction of water, where I_{420} and I_{365} are the emission intensities at 500 nm for the excitation at 420 and 365 nm respectively. This indicates that 420-nm excitation generates more 500-nm emission than does 365-nm excitation towards to higher percentage of water. (g) The position of the peak maxima of the two absorption bands (the blue and the red for shorter and longer wavelengths respectively) as a function of the mole fraction of water. There was no significant shift of the peak maxima except for the 420 nm band (red band) at the very high percentage of water, where it shows a blue shift.