**Supplementary Information**

# Tunable emissive carbon polymer dots with solvatochromic behavior for trace water detection and cell imaging

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## Methods

## 1. Reagents and materials

P-phenylenediamine (pPD), fluorescein, Poly(ethylene imine) (PEI$_{1800}$), Poly(vinylpyrrolidone) (PVP$_{10000}$), was purchased from Aladdin Biochemical Technology Co., Ltd. Ultra-dry ethanol, ultra-dry isopropanol, ultra-dry N, N-dimethylformamide, ultra-dry acetonitrile, ultra-dry acetone were purchased from J&K Scientific Co., Ltd. Rhodamine 101 (RB101) purchased from Sigma Co., Ltd. Ethylene glycol (EG), formamide (FA), methanol (MA), anhydrous ethanol (EA), n-propyl alcohol (NPA), isopropyl alcohol (IPA), n-butanol (NBA), n-amyl alcohol (NAA), dimethyl sulfoxide (DMSO),N, N-dimethyl formamide (DMF), acetonitrile (ACN), acetone (DMK), trichlorocarbon (TCM) , ethyl acetate (EAC), dichloromethane (DCM), toluene (TL) were purchased from China National Pharmaceutical Group Corporation. Poly(acrylic acid) (PAA) was purchased from Aldrich Co., Ltd. Poly(ethylene glycol) (PEG$_{6000}$) was purchased from Shanghai Hushi Laboratorial Equipment Co., Ltd. Carboxylic PEG acid (mPEG$_{750}$-COOH) was perchased from Biomatrik Inc. Ultra-pure water with a resistivity of 18.25 MΩ•cm$^{-1}$ was used in all experiments. Dulbecco's modified eagle medium (DMEM) was purchased from HYCEZMBIO Co.,Ltd. The reagents were all AR. The temperature and relative humidity of all experiments were about 20-25 °C and 50-60 %, respectively.

## 2. Instruments

The emission was obtained on fluorospectrophotometer (Fluorolog-3, Horiba Jobin Yvon Inc, France). The absorption spectrum was taken on ultra-violet spectrophotometer (UV-2550, Shimadzu, Japan). The transmission electron microscopy (TEM) and high-resolution TEM (HRTEM) graphs were obtained on transmission electron microscope (JEM-2100, JEOL, Japan). Fourier transform infrared spectra (FT-IR)

were taken on Fourier infrared spectroscopy (FTIR5700, Thermo, America). The X-ray photoelectron spectroscopy (XPS) was tested on X-ray Photoelectron Spectrometer (ESCALAB250Xi, Thermo Fisher Scientific, USA). The lifetime of CPDs was recorded using Steady State and Transient State Fluorescence Spectrometer (FLS1000, Edinburgh Instruments, United Kingdom). The confocal fluorescence images was obtained on a Confocal Fluorescence Microscope (Thorlabs, USA).

## 3. Lippert - Mataga model

The main factors affecting solvatochromism were generally divided into general and special solvent effect. The Lippert-Mataga equation was usually used to identify general and special solvent effects [1, 2]. Assuming that the solute-solvent system was a diatomic molecule, the difference of energy caused by the electronic transition between the excited state and the ground state, as well as the relationship between the polarity of the solvent and the Stokes shift, can be calculated by lippert-Mataga equation [2]:

$$\Delta v = \bar{v}_A - \bar{v}_F = 2(\mu_E - \mu_G)^2 \Delta f / hca^3 + constant \quad \text{(Eq. S1)}$$

Where $\Delta f = (\varepsilon - 1)/(2\varepsilon + 1) - (n^2 - 1)/(2n^2 + 1)$ is the directional polarizability of solvent determined by the dielectric constant and refractive index of the solvent.

## 4. Solvatochromic parameters

### 4.1. Solvent polarity parameter $E_T(30)$

The solvent polarity parameter $E_T(30)$ was proposed by Dimroth and Reichardt as an appropriate scale chosen to describe the solvent polarity [3]. It was obtained based on the experiments of pyridinol betaine dye 36 with negative solvatochromism as the probe molecule. And it was defined

as the molar electronic transition energy (ET), which was calculated by kcal/mol at room temperature (25 ℃) [4, 5]:

$$E_T(30) = hc\bar{v}_{max}N_{A} = (2.8591 \times 10^{-3})\bar{v}_{max}(cm^{-1}) = 28591/\lambda_{max}(nm) \quad \text{(Eq. S2)}$$

Where, $\lambda_{max}$ is the maximum absorption wavelength of pyridinol betaine dye in different solvents, $h$ is Planck's constant ($6.63 \times 10^{-34}$ J•s), $c$ is the speed of light ($3.0 \times 10^{10}$ cm/s), $N_A$ is Avogadro's constant.

The solvents were classified according to the value of $E_T(30)$. When the $E_T(30)$ value of the solvent is between 47 and 63 kcal/mol, it is protic solvent. When the $E_T(30)$ value is 40 to 47 kcal/mol, it is polar aprotic solvent, and when the $E_T(30)$ value is between 30 and 40 kcal/mol, it is a non-polar aprotic solvent.

## 4.2. Hydrogen bond index

Hydrogen bonding index (HB index) refers to the ability of a molecule to share a hydrogen atom with electro-negative groups on neighboring molecules [6]. Burell subdivided solvents into weak, medium, and strong hydrogen bonds solvents based on the strength of hydrogen bonding [7]. Alcohols belong to the strong hydrogen bond solvents. Ketones and esters belong to the medium hydrogen bond solvents, while simple hydrocarbon groups and chlorinated hydrocarbons belong to the weak hydrogen bond solvents.

## 4.3. Linear solvent energy relation

In 1976, Kamlet and Taft proposed a multi-parameter equation called linear solvation energy relation (LSER) for the quantitative treatment of solvent effects [8-10]. It is a generalized treatment of solvation effects,

which assumes that there are generally two kinds of solute-solvent interactions: nonspecific dipolarity/polarizability and specific hydrogen bond complex formation. In this work, according to the absorption and emission spectrum data, the three-parameter linear solvent-energy relation equation 3 was used [11]:

$$XYZ = XYZ_0 + s\pi^* + a\alpha + b\beta \qquad \text{(Eq. S3)}$$

Where $\pi^*$ is solvent polarizability, $\alpha$ is solvent hydrogen bond donor capacity, $\beta$ is hydrogen bond acceptor capacity. This equation is applicable to the influence of different solvents on the property of a single solute, such as absorption spectrum emission spectrum or other solvent-sensitive spectral parameters.

After calculating the values of the coefficients *s*, *a* and *b*, the linear solvation energy equation can be derived according to the data in Table 2-3 and Eq. 3:

$$E_{Abs} = 2.7723 - 0.1683\pi^* - 0.0316\alpha - 0.2290\beta, \; N=15 \qquad \text{(Eq. S4)}$$

$$E_{Em} = 2.5563 - 0.2107\pi^* - 0.2098\alpha - 0.2581\beta, \; N=15 \qquad \text{(Eq. S5)}$$

Where $E_{Abs}$ is absorption spectrum, $E_{Em}$ is emission energy respectively, $N$ is the number of different solvents used.


## 4.4. Radiative and nonradiative decay rate constants $K_r$ /$K_{nr}$

When the fluorophore is in the excited state, the excited molecule is unstable and can return to the ground state by radiative transition and non-radiative transition. The relative speed of the two processes plays a decisive role in the fluorescence quantum yield and fluorescence lifetime of the fluorophore. The change in quantum yield is due to the change in the non-radiative decay rate constant. Since the excitation coefficient and radiative decay rate constant are generally not very sensitive to the

polarity of the solvent, a reduction in quantum yield increases the nonradiative decay rate of nonpolar surface solvents [12]. The decay rate constants of radiation ($K_r$) and non-radiation ($K_{nr}$) can be calculated using the experimentally calculated values of quantum yield ($\varphi$) and fluorescence lifetime ($\tau$). These rate constants can be calculated by the following formulas [13]:

$$K_r = \varphi/\tau \qquad \text{(Eq. S6)}$$

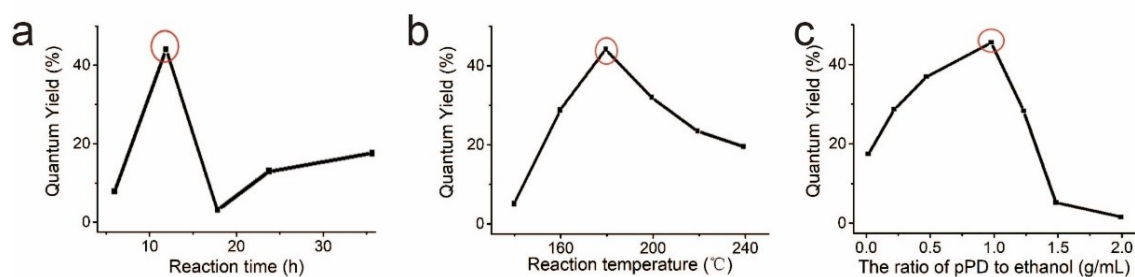$$K_{nr} = (1 - \varphi)/\tau \qquad \text{(Eq. S7)}$$



Figure S1 Investigation for the synthesis conditions of CPDs under different reaction time (a), reaction temperature (b) and reaction precursor ratio(c).

Table S1 Relative parameters of solvents. Serial numbers 1-8 are protic solvents and 9-16 are aprotic solvents. $\Delta f$: directional polarization; $E_T(30)$: solvent polarity parameter; HB index: hydrogen bond coefficient; $\pi^*$: solvent polarizability; $\alpha$: hydrogen bond donor capacity; $\beta$: Hydrogen bond receptor capacity.

| No. | Solvents | Δf | $E_T(30)$ [5] | HB index | Kamlet-Taft Parameters [14] | | |
|-----|----------|-----|---------------|----------|-----|-----|-----|
| | | | | | π* | α | β |
| 1 | EG | 0.2745 | 56.3 | 20.6 | 0.92 | 0.90 | 0.52 |
| 2 | FA | 0.2822 | 55.8 | - | 0.97 | 0.71 | 0.48 |
| 3 | MA | 0.3086 | 55.4 | 18.7 | 0.60 | 0.93 | 0.62 |
| 4 | EA | 0.2887 | 51.9 | 18.7 | 0.54 | 0.83 | 0.77 |
| 5 | NPA | 0.2741 | 50.7 | 18.7 | 0.52 | 0.78 | 0.80 |
| 6 | NBA | 0.2619 | 49.7 | 18.7 | 0.47 | 0.79 | 0.88 |
| 7 | NAA | 0.1987 | 49.1 | 18.7 | - | - | - |
| 8 | IPA | 0.2729 | 48.4 | - | 0.48 | 0.76 | 0.95 |
| 9 | DMSO | 0.2630 | 45.1 | 7.7 | 1.00 | 0.00 | 0.76 |
| 10 | DMF | 0.2744 | 43.2 | 11.7 | 0.88 | 0.00 | 0.69 |
| 11 | ACN | 0.3046 | 45.6 | 6.3 | 0.75 | 0.19 | 0.31 |
| 12 | DMK | 0.2846 | 42.2 | 9.7 | 0.71 | 0.08 | 0.48 |
| 13 | TCM | 0.1459 | 39.1 | 1.5 | 0.58 | 0.44 | 0.00 |
| 14 | EAC | 0.2002 | 38.1 | 8.4 | 0.55 | 0.00 | 0.45 |
| 15 | DCM | 0.2185 | 40.7 | 1.5 | 0.73 | 0.30 | 0.00 |
| 16 | TL | 0.0135 | 33.9 | 4.5 | 0.54 | 0.00 | 0.11 |

Table S2 Detailed spectral parameters of the CPDs in different solvents. Serial numbers 1-8 are protic solvents and 9-16 are aprotic solvents. Δf: directional polarization; Absorption: maximum Absorption peak wavelength; Emission: maximum Emission peak wavelength; Stokes shift: Stokes shift; QY: quantum yield; τ: fluorescence lifetime.

| No. | Solvents | Δf | Absorption (nm) | Emission (nm) | Stokes shift | QY | τ (ns) |
|-----|----------|-----|-----------------|---------------|--------------|-----|--------|

| | | | | | (nm) | | |
|---|---|---|---|---|---|---|---|
| 1 | EG | 0.2745 | 503 | 608 | 3433.35 | 0.168 | 6.17 |
| 2 | FA | 0.2822 | 497 | 596 | 3342.20 | 0.192 | 6.95 |
| 3 | MA | 0.3086 | 501 | 598 | 3237.67 | 0.243 | 7.36 |
| 4 | EA | 0.2887 | 502 | 598 | 3197.91 | 0.323 | 8.28 |
| 5 | NPA | 0.2741 | 505 | 598 | 3071.73 | 0.233 | 8.47 |
| 6 | NBA | 0.2619 | 505 | 598 | 3063.90 | 0.271 | 8.64 |
| 7 | NAA | 0.1987 | 506 | 598 | 3040.44 | 0.268 | 8.82 |
| 8 | IPA | 0.2729 | 507 | 594 | 2888.85 | 0.421 | 9.37 |
| 9 | DMSO | 0.2630 | 510 | 578 | 2306.81 | 0.273 | 12.03 |
| 10 | DMF | 0.2744 | 500 | 567 | 2363.32 | 0.289 | 10.53 |
| 11 | ACN | 0.3046 | 484 | 550 | 2479.34 | 0.251 | 5.76 |
| 12 | DMK | 0.2846 | 489 | 550 | 2268.08 | 0.239 | 9.37 |
| 13 | TCM | 0.1459 | 485 | 541 | 2134.27 | 0.377 | 6.73 |
| 14 | EAC | 0.2002 | 482 | 540 | 2228.37 | 0.316 | 6.65 |
| 15 | DCM | 0.2185 | 470 | 531 | 2444.20 | 0.448 | 4.52 |
| 16 | TL | 0.0135 | 476 | 526 | 1997.00 | 0.173 | 2.32 |
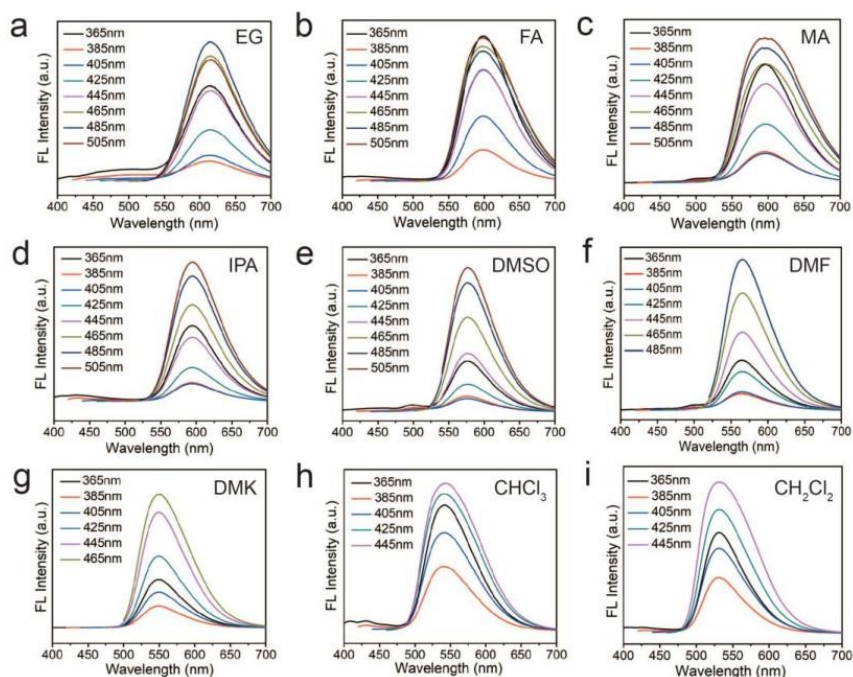
Figure S2 The fluorescence spectra of CPDs in (a) EG, (b) FA, (c) MA, (d) IPA, (e) DMSO, (f) DMF, (g) DMK, (h) CHCl₃, (i) CH₂Cl₂ under various excitation wavelengths.
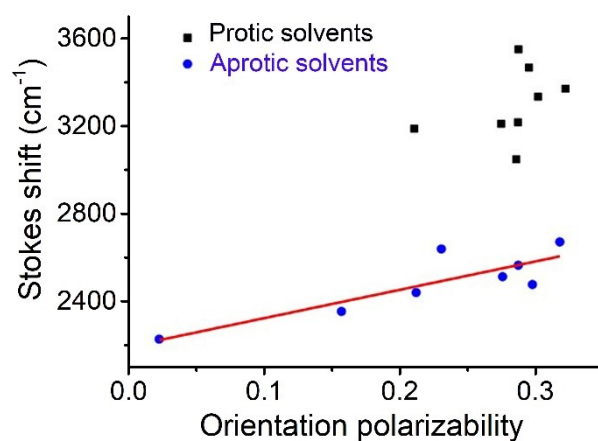


Figure S3 Lippert-Mataga plot between the orientation polarizability of solvents and the Stokes shift of the CPDs in protic solvents (black point) and aprotic solvents (blue point).

Table S3 Radiation and non-radiation decay rate constants of CPDs.

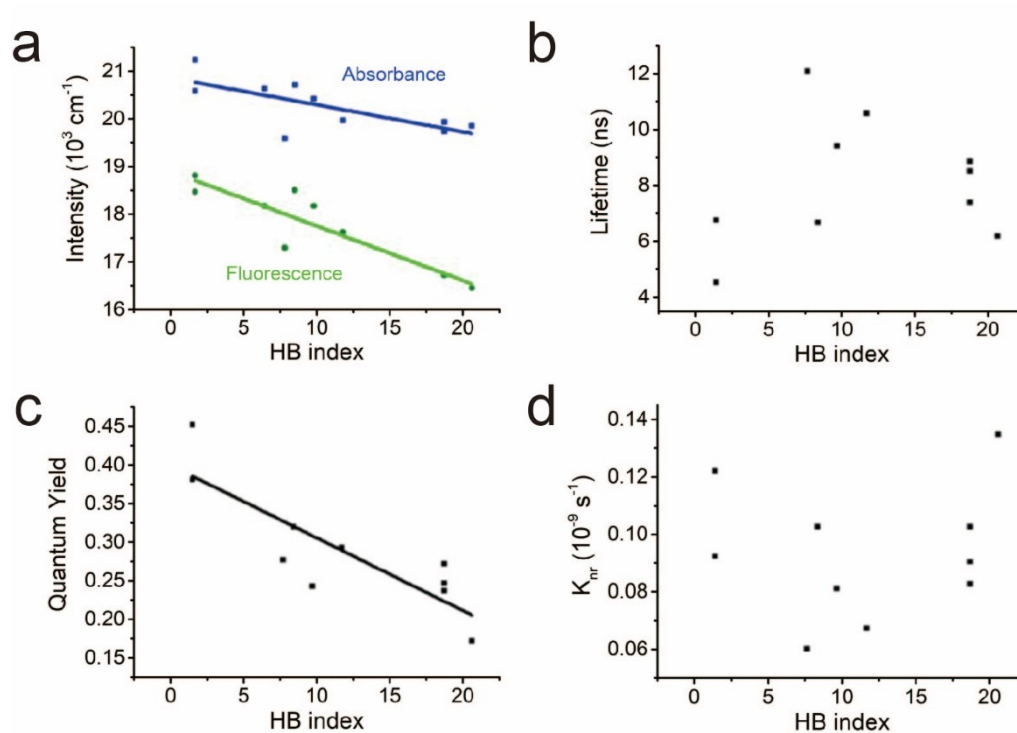| Protic solvents | EG | FA | MA | EA | NPA | NBA | NAA | IPA |
|---|---|---|---|---|---|---|---|---|
| kr (10-9s-1) | 0.027 | 0.028 | 0.033 | 0.039 | 0.028 | 0.031 | 0.030 | 0.045 |
| knr (10-9s-1) | 0.145 | 0.116 | 0.103 | 0.082 | 0.091 | 0.084 | 0.083 | 0.062 |
| Aprotic solvents | DMSO | DMF | ACN | DMK | TCM | EAC | DCM | TL |
| kr (10-9s-1) | 0.023 | 0.027 | 0.044 | 0.026 | 0.056 | 0.046 | 0.099 | 0.075 |
| knr (10-9s-1) | 0.060 | 0.068 | 0.130 | 0.081 | 0.093 | 0.1-3 | 0.122 | 0.356 |



Figure S4 Relationship between the spectral parameters of the CPDs and the solvents HB index: (a) absorption and emission. (b) lifetime. (c) QY. (d) non-radiative decay rate constant.
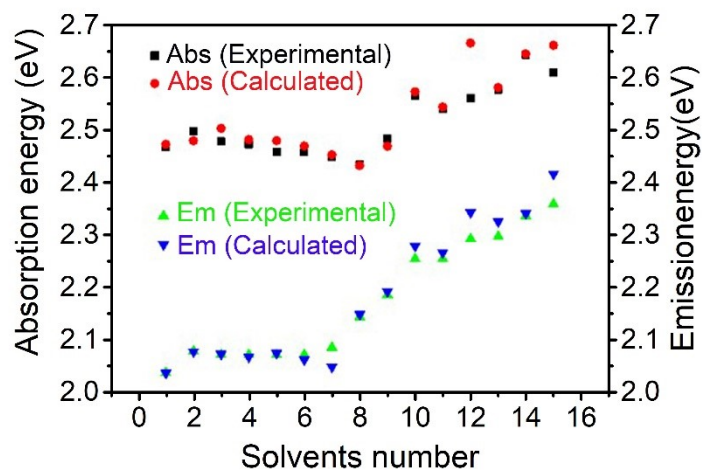
Figure S5 Kamlet-Taft plots for CPDs: the experimental data and the calculated data basically match.

Table S4 Detection of trace water in different organic solvents.

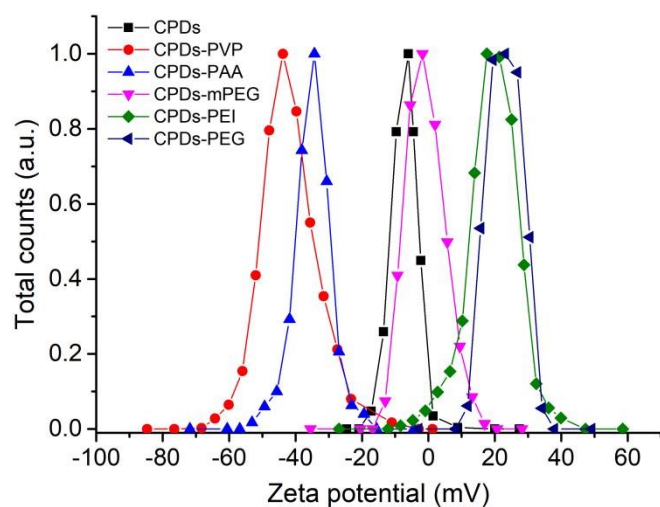| Solvents | Regression equation | $R^2$ | linear range (v/v) | LOD (v/v) |
|----------|---------------------|-------|---------------------|-----------|
| EA | $\dfrac{F_0 - F}{F_0} = 0.01747x + 0.01027$ | 0.990 | 0.1%-10% | 0.085% |
| IPA | $\dfrac{F_0 - F}{F_0} = 0.02258x + 0.00571$ | 0.989 | 0.1%-7% | 0.079% |
| DMF | $\dfrac{F_0 - F}{F_0} = 0.02389x + 0.02069$ | 0.987 | 0.1%-10% | 0.094% |
| ACN | $\dfrac{F_0 - F}{F_0} = 0.08513x + 0.00849$ | 0.984 | 0.1%-7% | 0.089% |

Figure S6 The ζ-potential for CPDs and CPDs-polymer composites.
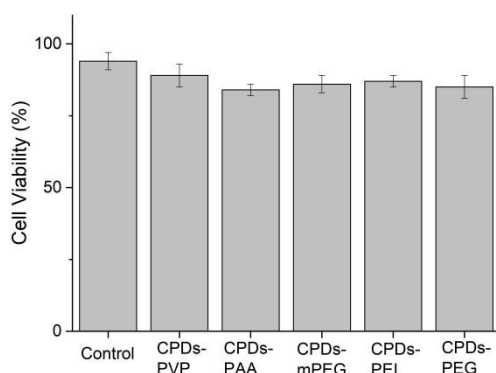


Figure S7 The cytotoxicity assay of different polymer modified CPDs, respectively.

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