The Cationic Dye Basic Orange 21 (BO21) as a Potential Fluorescent Sensor

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Electronic Supplementary Information

Theoretical Background: Fluorescence Polarization, Lifetime and Quantum Yield

Fluorescence polarization: Even though low concentration fluorescent molecules in solution are isotropically distributed, excited molecules are not when they are directionally photoselected by a linearly polarized excitation beam. Consequently, the right-angle detected fluorescence will be polarized. The fluorescence polarization p is defined as:

$$p = \frac{I_{||} - G \cdot I_{\perp}}{I_{||} + G \cdot I_{\perp}} \tag{S1}$$

where $I_{||}$ and $I_{\perp}$ are the fluorescence intensities measured through an analyzer polarizer when the plane of polarization is parallel and perpendicular, respectively, to the electric field vector of the excitation light. The unevenness in the detection preference of the instrument to the parallel and perpendicular components is corrected by the factor G.[S1]

The measured fluorescence polarization p of relatively small and spherical molecules hosted in a viscous solution is given by Perrin's equation:[S2]

$$\left(\frac{1}{p} - \frac{1}{3}\right) = \left(\frac{1}{p_0} - \frac{1}{3}\right)\left(1 + \frac{\tau}{\tau_c}\right) \tag{S2}$$

where p_0 is the fundamental fluorescence polarization, τ is the fluorophore fluorescence lifetime (FLT), and $\tau_c = \frac{V\eta}{RT}$ is the rotational correlation time, where $V = \frac{M_W}{\rho}$ (M_W is the molecular weight and ρ is the volumetric density, assumed to be 1 g/mL) is the molecular hydrodynamic volume, η is the viscosity of the hosting media, and T is the absolute temperature. When the fluorophore transition dipole absorption and emission moments are parallel and the fluorophores behave as if they were a frozen, non-interacting gas in the hosting solution, then $p_0 \rightarrow$ ½.[S2] As can be seen from Eq. S2,

when $\tau/\tau_c \to 0$, $p \to p_0$, and when $\tau/\tau_c \to \infty$, $p \to 0$, which in practice happens in hosting media with very high (glycerol) and very low (water) viscosities, respectively. It can be shown that for randomly distributed motionless fluorophores, the fundamental fluorescence polarization is $-\frac{1}{3} \le p_0 \le \frac{1}{2}$ depending on the angle between the absorption and emission transition moments of the fluorophore.[S2]

Fluorescence lifetime and quantum yield: The ratio between the number of photons emitted and the number of photons absorbed is defined as the fluorescence quantum yield ϕ. For practical reasons, the quantum yield of BO21 was assessed in this study with respect to a reference fluorophore (r) with a known quantum yield:[S3]

$$\phi = \phi_r \left(\frac{I}{I_r}\right)\left(\frac{OD_r}{OD}\right)\left(\frac{n}{n_r}\right)^2 \tag{S3}$$

where n and n_r are the indices of refraction of BO21 solution and of the reference, respectively, and OD is the optical density. To avoid corrections due to different values of absorbance, the sample and the reference are excited at the same wavelength where they both have equal absorption.

The quantum yield ϕ is related to the measured fluorescence lifetime τ_M and the natural lifetime τ_0:[S4]

$$\tau_M = \tau_0 \cdot \phi \tag{S4}$$

Thus, by measuring τ_M and ϕ one can calculate τ_0. In several cases, τ_0 can also be estimated by the Stickler-Berg (SB) relation:[S5]

$$\frac{1}{\tau_0} = 2.88 \times 10^{-9} \cdot n_{solv}^2 \cdot \frac{\int I(v)dv}{\int v^{-3} I(v)dv} \cdot \int \frac{\varepsilon(v)}{v} dv \equiv 2.88 \times 10^{-9} \cdot n_{solv}^2 \cdot F \cdot A \tag{S5}$$

where n_{solv} is the index of refraction of the hosting solvent at the chief emission wavelength of the fluorophore, $I(v)$ is the emission intensity, $\varepsilon(v)$ is the molar absorption coefficient, v is the wavenumber (in cm^{-1}), and the F and A are the

fluorescence ratio integral and the absorption integral, respectfully. However, Eq. S5 does not address situations where the fluorophore (a) interacts with the hosting media, (b) is built of substituted aromatic components or behaves similarly, (c) is quenched to a level that cannot be ignored, (d) the $S_0 \rightarrow S_1$ transition is relatively weak ($\varepsilon_{max} <$ 8000), or (e) the first excited singlet state is negligibly deformed.[S5] Based on our experience, these principal limitations do not hold for BO21 ($\varepsilon_{max} \cong 60000$), and hence using the SB equation for BO21 in water should yield a correct value for τ_0. From the relation between the measured lifetime and the rate constants for radiative and nonradiative processes:[S4a]

$$\tau_M = \frac{1}{K_r + K_{nr}} \tag{S6}$$

the inverse relation between the natural life and its rate constant $\left(\frac{1}{K_r} = \tau_0\right)$ and the relation in Eq. S4, one gets:

$$\tau_M = \phi \cdot \tau_0 = \frac{\phi}{K_r} \Rightarrow K_r = \frac{\phi}{\tau_M} \tag{S7}$$

Substituting Eq. S7 into Eq. S6 one can get the rate constant of nonradiative processes from the measured quantum yield ϕ and τ_M:

$$\tau_M = \frac{1}{\frac{\phi}{\tau_M} + K_{nr}} \Rightarrow K_{nr} = \frac{1}{\tau_M}(1 - \phi) \tag{S8}$$

Computational Methods:

All calculations were performed using Gaussian09 Revision E.01[S6] or Orca 3.0.3;[S7] double hybrid calculations were done with the latter and all others with the former. Geometries were optimized using the hybrid-meta-GGA version[S8] of the Hamprecht–Cohen–Tozer–Handy[S9] functional (τHCTHhyb) with the def2-SVP (double-ζ plus

polarization quality) basis set.[S10] The functional used was chosen based on its performance for TDDFT (*vide infra*) but is also reasonable for ground state calculations. Energies were calculated using Kozuch and Martin's dispersion corrected (with an empirical dispersion correction,[S11] specifically the third version of Grimme's dispersion[S12] with Becke–Johnson dampening[S11,13] – D3BJ), spin component scaled (*i.e.*, an SCS[S14]-MP2[S15]-like correlation contribution), double hybrid (DSD) functional, incorporating the PBE exchange[S16] and the Becke-95 (B95) correlation[S17] functionals (i.e., DSD-PBEB95)[S18] with the def2-TZVP (polarized valence triple-ζ) basis set and the resolution of identity-chain of spheres exchange (RIJCOSX) approximation[S19] to increase the speed of the calculations. Excitation energies were calculated using time-dependent DFT (TDDFT).[S20] The τHCTHhyb functional was chosen based on results by Isegawa *et al.*:[S21] this functional has the lowest MUEs for overall valence excitations and for azabenzenes, which are the closest examples in that study to BO21. While only the first excitation (root) is of interest, ten roots were considered. These calculations used the PBE0/def2-SVP optimized geometries and the def2-TZVP basis set, except where noted otherwise. To calculate the fluorescence (LE) and TICT (*vide infra*) states, the relevant excited state geometries were optimized with the def2-SVP basis set, and the TDDFT energy calculations were repeated with the def2-TZVP basis set. Bulk solvent effects were approximated by using a polarizable continuum model (PCM),[S22] specifically the integral equation formalism model (IEF-PCM)[S22-23] with water as the solvent as in the experiments. Specifically, Truhlar's empirically parameterized version Solvation Model Density (SMD) was used.[S24] The SMD model was used in all calculations, including geometry optimizations. All potential energy surfaces (PES) are relaxed scans, where one dihedral angle (θ) is scanned and at each value all other coordinates are optimized. PESs are done at the τHCTHhyb/def2-SVP level of theory

with the Kohn-Sham (KS) and TDDFT surfaces optimized separately. For interpretative purposes, Löwdin charges[S25] are presented determined at the SMD-τHCTHhyb/def2-TZVP or SMD-TD-τHCTHhyb/def2-TZVP level of theory. The natural transition orbitals (NTOs)[S26] are used to help determine the character of key transitions from the TDDFT calculations. The contributions of given fragments of the molecule to each NTO was done using a Hirshfeld weighting scheme[S27] using MULTIWFN version 3.3.9.[S28]

Measurement of Fluorescence Polarization:

The fluorescence polarization was calculated from the same measurements using Eq. S1. The correction factor $G = I_{HV}/I_{HH}$, where I_{HV} and I_{HH} are the parallel and perpendicular fluorescence intensities when the excitation is performed with horizontally polarized light (*i.e.*, the electric vector field vibrates within the plane of measurement defined by the directions of the excitation beam and emission detection).[S3b]

Fluorescence lifetimes were measured by a time-correlated single-photon counting method by two different apparatuses:

a. ISS ChronosBH (ISS, Champaign, IL, USA) time correlated single photon counting (TCSPC) fluorimeter with a T-geometry, a 472 nm laser diode pulsing at a 20 MHz repetition rate, a SPC130 acquisition card, a detector PMC100-40 from Becker & Hickl GmbH (Berlin, Germany), and multiple bandpass filters and a long pass optical filter.

b. An in-house assembled system, which includes a femtosecond Ti-sapphire laser (Chameleon, Coherent, Santa Clara, CA, USA) for excitation at 465 nm. The emission at 520 nm was selected (emission bandwidth 20 nm) by a double monochromator (DIGIKROM CM112, Albuquerque, NM, USA) and

detected by a Hamamatsu R9880U-210 fast photomultiplier. For data collection, a single-photon counting board SPC 630, Becker and Hickel GmbH, Berlin, Germany) was used fed via a preamplifier (HFAC-26DB 0.1UA, Brookline, MA, USA) triggered by a photodiode (PHD-400N). Measurements were performed at 23 °C.

Table S1. Summary of spectroscopic properties of BO21 in aqueous glycerol solutions of varying concentrations: quantum yield (ϕ), peak absorption and emission wavelengths (λ_{abs} and λ_{em} in nm), experimentally measured lifetime (τ_{exp}), and lifetimes according to the Strickler-Berg (τ_{SB}, Eq. S5, ns) and Perrin (τ_{Perrin}, Eq. S2, ns) relations.

%glycerol	ϕ	λ_{abs}	λ_{em}	τ_{exp}	τ_{SB}	τ_{Perrin}
0	0.002	484	514		0.007	0.007
20	0.003	487	519		0.008	0.007
40	0.005	490	519		0.015	0.012
60	0.010	493	522			0.031
70	0.016	494	523		0.039	0.044
80	0.027	493	523		0.054	0.079
85	0.028			0.18	0.088	0.101
90	0.053	496	523	0.21	0.153	0.206
95	0.090			0.3	0.285	0.460
100	0.115	497	523	0.43	0.306	0.377

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