

# Multiwavelength Ratiometric Fluorescence Sensing

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## Two channels of information from a single dye

Intermolecular interactions involve physical forces of different origin. The goal of many sensing and imaging technologies is to characterize these interactions and their dynamics from a single dye. To achieve such a goal we propose the use of dyes exhibiting ground-state and excited-state transformations that switch fluorescence between several emission bands. These transformations can be influenced by properties of the environment of the dye such as polarity, viscosity, electronic polarizability, H-bonding ability, temperature, pressure, pH, analyte concentrations, etc. We show that curve-fitting based on deconvolution into log-normal components promises a reliable physical modeling of the probe response to intermolecular interactions [1]. Two channels of information are derived, based on independent detection of ground-state and excited-state equilibria (figure 1).

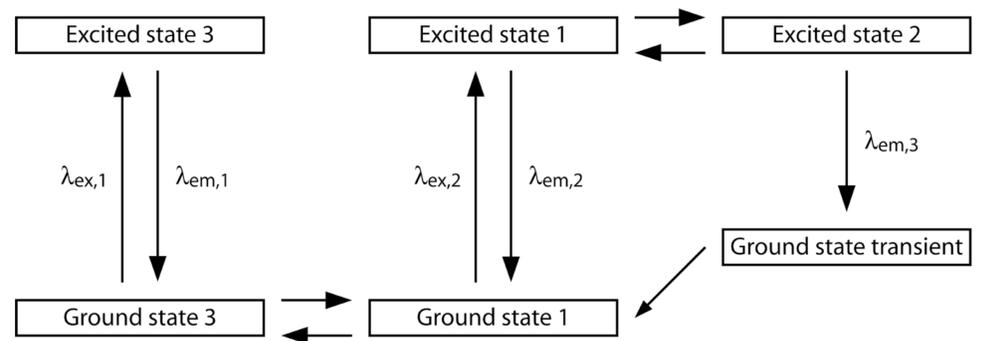


Figure 1: A dye exhibiting two equilibria, one in the ground state and one in the excited state. By measuring the emission spectra at different excitation wavelengths we can recover the state of these equilibria, and therefore characterize the properties that influence them.

## Two-dimensional fluorescence deconvolution

To reliably measure the state of the two equilibria for closely spaced bands, we perform two-dimensional deconvolution of the excitation-emission landscape. While just fitting three closely spaced fluorescence emission bands in such a way would be prone to error, a two-dimensional deconvolution allows us to use the shift in excitation spectrum in order to make the separation (figure 3). The fluorescence of each of the emissive species is modeled using two Siano-Metzler [2] asymmetric log-normal functions (figure 2): one for excitation and one for emission. Because two of the species share a single ground state, the shapes of their excitation bands are kept equal. Altogether, the two excitation and three emission bands have a total of 18 parameters:

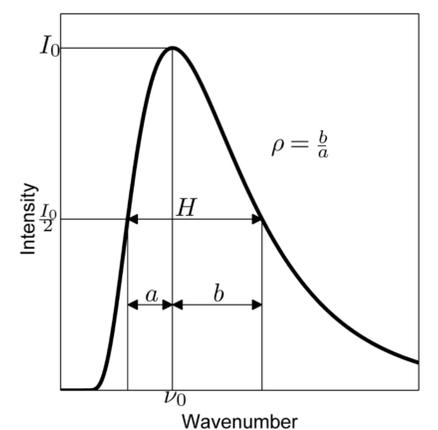


Figure 2: Parameters of the Siano-Metzler [2] asymmetric log-normal function.

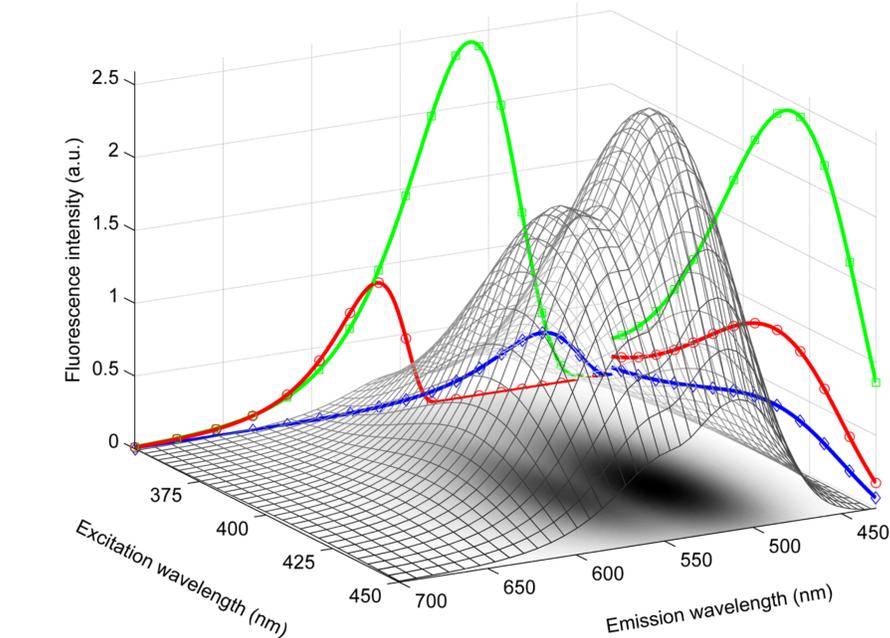


Figure 3: Three-band deconvolved 2D fluorescence spectrum in the case of strong overlap between individual bands. The combined spectrum is represented as a 2D surface and mesh, while the individual emission and excitation bands (at their respective excitation and emission maxima) are projected onto the sides. The data are for FE [3] dye in octanol, with state 1 ( $N^*$ , blue diamonds), 2 ( $T^*$ , red circles) and 3 ( $H-N^*$ , green squares) bands.

## Deconvolution in mixtures and solvents

We verified the deconvolution procedure by unmixing FE [3] dye spectra obtained in various mixtures of ethyl acetate and water. With FE, the excited state equilibrium measures solvent polarity, while the hydrogen bond donor capacity determines the ground state equilibrium. Figure 4 shows that adding water greatly increases the HBD ability while only slightly affecting polarity. By factoring the two equilibria, we can accurately measure the excited state equilibrium in the presence of a second ground state. We used this to measure the solvent polarity of protic solvents with FE. Whereas in previous data [3] the protic solvents required a separate calibration, our analysis allows them to be fit with the same curve as the aprotic solvents (figure 5). We have applied this technique to the study of  $\alpha$ -synuclein aggregation [4], see poster 67 [5].

$$\text{alogn}(v_0, H, \rho, I_0, v) = I_0 e^{-\left(\frac{\ln 2}{(\ln \rho)^2}\right) \left[\ln\left(\frac{v-v_0}{H}\right) \left(\frac{\rho^2-1}{\rho}\right)\right]^2}$$

$$I(\lambda_{\text{ex}}, \lambda_{\text{em}}) = \sum_{b \in \text{bands}} \text{alogn}\left(v_{0,\text{em}}^b, H_{\text{em}}^b, \rho_{\text{em}}^b, \text{alogn}\left(v_{0,\text{ex}}^b, H_{\text{ex}}^b, \rho_{\text{ex}}^b, I_0^b, \frac{10^7}{\lambda_{\text{ex}}}\right), \frac{10^7}{\lambda_{\text{em}}}\right)$$

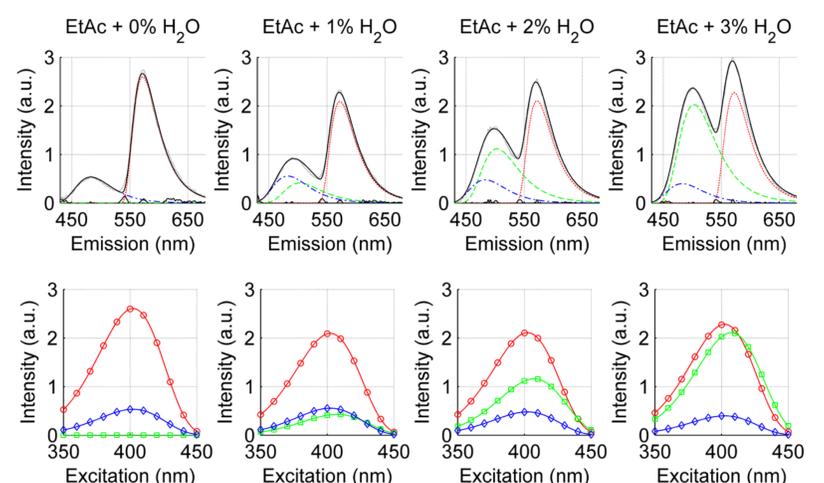


Figure 4: Emission and virtual excitation spectra of FE dye in various mixtures of ethyl acetate and water. The ratio between excited states 1 ( $N^*$ , blue) and 2 ( $T^*$ , red) is constant, while state 3, ( $H-N^*$ , green), showing the second state of the ground state equilibrium is increasing. Virtual excitation spectra were created by plotting the band intensities at their respective emission maxima.

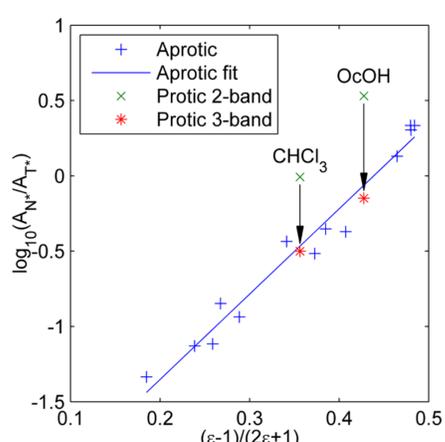


Figure 5: Ratio of the excited state equilibrium of FE as a function of solvent polarity function  $f(\epsilon)$ , showing a linear relationship. The solvents are (from left to right): hexane, toluene, carbon disulfide, thiophene, *d*-*n*-butyl ether, anisole, chloroform, bromobenzene, ethyl acetate, tetrahydrofuran, octanol, acetone, DMF, acetonitrile, and DMSO (data from [3] kindly provided by Dr. AS Klymchenko). The values for octanol (OcOH) and chloroform ( $\text{CHCl}_3$ ) are given for both the original 2-band and improved 3-band deconvolution, the shift in results being indicated by arrows.

## References

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