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Recovery of Lifetime Distributions from Frequency-Domain Fluorometry Data by Means of the Quantified Maximum Entropy Method

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The new quantified version of the maximum entropy method allows one to recover lifetime distributions with a precise statement of the accuracy of position, surface, and broadness of peaks in the distribution. Applications to real data (2,6-ANS in aqueous solutions of sodium dodecyl sulfate micelles of β-cyclodextrin) are presented.

KEY WORDS: Maximum entropy method; frequency-domain fluorometry; lifetime distributions.

Time-resolved fluorescence spectroscopy is widely used in the physical, chemical, and biological sciences to get information on the dynamics of molecular, macromolecular, or supramolecular systems. In most cases the fluorescence decay curves can be satisfactorily described by a sum of discrete exponentials and the physical significance of the preexponential factors and decay times is well assigned. But in complex systems such as proteins, micellar solutions, vesicles, or membranes, continuous decay-time distributions are sometimes anticipated to best account for the phenomena. It should be emphasized that reliable interpretation of experiments in terms of a sum of discrete exponentials and/or a continuous distribution requires an approach without a priori assumption of the distribution shape. In this respect, the maximum entropy method (MEM) has been successfully applied to the analysis of data in time-domain fluorometry [1,2] and in frequency-domain fluorometry [3].

The new quantified version of MEM includes a precise statement of the accuracy of quantities of interest, i.e., position, surface, and broadness of peaks in the lifetime distribution. The distributions are recovered by using an automatic stopping criterion for successive iterates which is based on a Gaussian approximation of the likelihood. The model $m(\tau)$ of the prior guess for the structure of the distribution $f(\tau)$ (formula 12 in Ref. 4). In absence of any prior information it is set to be a flat distribution since all lifetimes have an identical probability to be present.

Applications to real data obtained with our multifrequency phase-modulation fluorometer [4] are now presented. In aqueous solutions of sodium dodecyl sulfate (SDS) micelles, 2,6-ANS (2-anilinonaphthalene-6-sulfonate) probes the outer-core region of the micelles, and the microheterogeneity of solubilization sites results in a distribution of lifetimes, as first reported by Huang and Bright [5]. Analysis of our data at 25 and 60°C by MEM confirms the existence of such a distribution. To take into account the possible existence of free ANS molecules in the aqueous phase, analysis has been performed with an additional single-exponential component corresponding to the lifetime of ANS in water, i.e., 0.38 ns (25°C) and 0.25 ns (60°C). The recovered distributions are shown in Fig. 1 and their characteristics are reported in Table I. By using a nonlinear least-squares method, Huang and Bright found that their data are best

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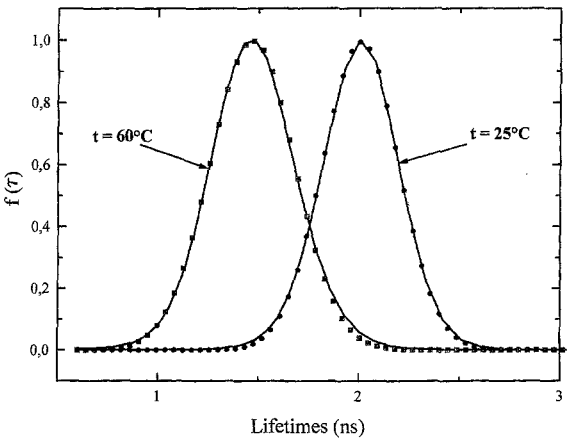

Fig. 1. Recovered lifetime distributions (solid lines) for ANS in an aqueous solution containing micelles of sodium dodecyl sulfate (0.1 *M*) and 2,6-ANS (10^{-5} *M*) at 25 and 60°C. Excitation at 325 nm (He–Cd laser); observation with a Balzers bandpass filter at 460 nm. Number of modulation frequencies: 24. Analysis by MEM has been performed with an additional single-exponential component (free ANS) at 0.38 nm (25°C) and 0.25 nm (60°C). Analysis with 100 image points (log scale) from 0.6 to 25 ns. Best fits of the recovered distributions by a Gaussian (●, ■).

Table I. Center and Width of the Recovered Distributions by MEM for ANS in Aqueous Solutions of SDS micelles

T(°C)	τ_0 (ns)[a]	Center (ns)	Width (ns)	χ^{2b}
25	—	1.98 ± 0.04	0.23 ± 0.12	0.953
25	0.38	1.98 ± 0.03	0.20 ± 0.11	0.952
60	—	1.44 ± 0.03	0.22 ± 0.07	0.642
60	0.25	1.44 ± 0.02	0.22 ± 0.06	0.643

[a]Additional single-exponential component.
[b]Experimental errors: 0.1° on phase shift and 0.001 on modulation ratio.

fit by a Lorentzian distribution. In contrast, analysis of our data by MEM shows that the recovered distributions have a Gaussian shape (see Fig. 1).

2,6-ANS has also been incorporated into the cavity of β-cyclodextrin in aqueous solution. Evidence for lifetime distributions in the 1:1 inclusion complex was previously reported by Bright and co-workers [6] and assigned to an array of cyclodextrin-cavity environments. Analysis of their data by a nonlinear least-squares method with a priori assumption of either a Gaussian or a Lorentzian distribution did not allow them to distinguish between these distributions because their chi-square values were too similar. Our data have been

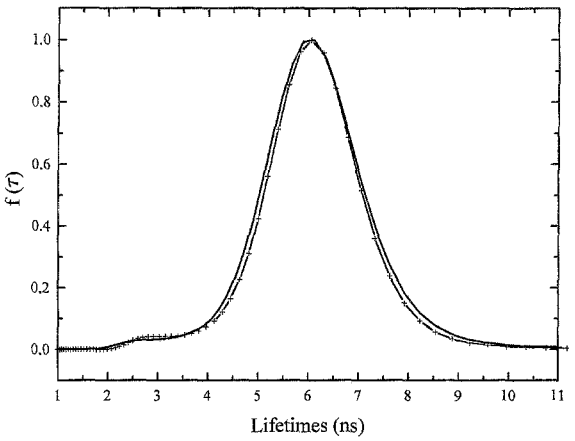

Fig. 2. Recovered distribution for an aqueous solution of 2,6-ANS (10^{-5} *M*) and β-cyclodextrin (10^{-3} *M*) at 25°C. Excitation and observation conditions similar to those for Fig. 1. Analysis with 150 image points from 0.1 to 25 ns (log scale) without an additional single exponential component (——). Analysis with 85 image points from 1 to 25 ns (log scale) with an additional single exponential component (+++++) at 0.38 ns.

analyzed by MEM (Fig. 2): Again, the recovered distribution turns out to be closer to a Gaussian than to a Lorentzian (not shown) in both analyses with and without an additional single-exponential component at 0.38 ns (possible free ANS).

In conclusion, the quantified MEM offers a powerful tool for the analysis of complex decays capable of handling both continuous and discrete lifetime distributions in a single analysis. The absence of an *a priori assumption on the shape of a distribution* is essential because a satisfactory fit of the experimental data by a nonlinear least-squares method with assumption of a Gaussian or a Lorentzian distribution only indicates that the assumed distribution is compatible with the experimental data, and does not demonstrate that such a distribution is the most probable one.

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