Supporting Information (SI)

Using the fluorescence red edge effect to measure the local dynamics of lyophilized sugar glasses and protein formulations, and assess long-term product stability

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Derivation of Equation 8:

Using the time-resolved fluorescence method, the spectral relaxation can be described by a Debye-type decay function, Φ(t):

$$\Phi(t) = \exp\left(-t/\tau_R\right) = \frac{\nu(t) - \nu(\infty)}{\nu(0) - \nu(\infty)} \qquad \text{(S1)}$$

where ν(t) is the first moment average of emitted fluorescence frequency (ν = c/λ) at time t. ν(0) and ν(∞) correspond respectively to the first moment averages of emitted fluorescence frequency immediately after excitation and at sufficiently long time that the excited-state solvent configuration is at equilibrium. τ_R is the relaxation time.

The first moment average of a corresponding steady-state emission spectra ($\bar{\nu}$) is given by the integral average of ν(t) over the intensity decay:

$$\bar{\nu} = \frac{\int_0^{\infty} \nu(t)\left[\exp\left(-t/\tau_F\right)\right]dt}{\int_0^{\infty} \nu(t)dt} \tag{S2}$$

where τ_F is the fluorescence lifetime.

Substitution of Equation S1 into S2 yields:

$$\frac{\bar{\nu} - \nu(\infty)}{\nu(0) - \nu(\infty)} = \frac{\tau_R}{\tau_R + \tau_F} \tag{S3}$$

Using the same formalism, we calculated the relaxation time from the red edge measurements by normalizing the spectral difference to the maximal red-edge effect at the lowest temperatures:

$$\frac{\bar{\nu}^{Main} - \bar{\nu}^{Edge}}{\bar{\nu}_0^{Main} - \bar{\nu}_0^{Edge}} = \frac{\tau_R}{\tau_R + \tau_F} \tag{S4}$$

where $\bar{\nu}^{Main}$ and $\bar{\nu}^{Edge}$ correspond to the first moments of fluorescence signals excited at 532 nm and 566 nm at a particular temperature, respectively. The subscript “0” in $\bar{\nu}_0^{Main}$ and $\bar{\nu}_0^{Edge}$ denotes first moments at the lowest temperature.