Enzyme kinetics in acoustically levitated droplets of supercooled water: a novel approach to cryoenzymology

David D. Weis[*†] *and Jonathan D. Nardozzi*[‡]

Department of Chemistry, Skidmore College

815 North Broadway, Saratoga Springs, NY 12866-1632

[*]Corresponding author.

[†]Present address: Department of Chemistry, MSC03 2060, 1 University of New Mexico, Albuquerque, NM 87131-0001, dweis@unm.edu, (505) 277-2902 (fax).

[‡]Present address: Department of Biochemistry and Molecular Biology, SUNY Upstate Medical University, Syracuse, NY 13210.

ABSTRACT A two-wavelength method to separate individual fluorescence emission intensities in a two-component mixture when the emission spectra overlap is derived.

Supporting Information

Since the emission by the substrate, MUP, overlaps with emission by the product, coumarin 4, the MUP emission must be subtracted from the emission at the coumarin 4 wavelength. The emission by the resorufin internal standard did not overlap with either MUP or coumarin 4. Let λ_M, λ_c, and λ_r, represent the emission wavelengths for MUP, coumarin 4, and resorufin, respectively, and let *E*, with the appropriate subscript, represent the fluorescence emission intensity at these wavelengths. Since the resorufin emission is well-resolved from MUP and coumarin 4, we may assume that the fluorescence emission intensity at the MUP and coumarin 4 emission wavelengths is the sum of emission by the two components

$$E_{\lambda_M} = E_{\lambda_M}^{(M)} + E_{\lambda_M}^{(c)}$$

$$E_{\lambda_c} = E_{\lambda_c}^{(M)} + E_{\lambda_c}^{(c)}$$

where the superscripts (M) and (c) denote emission by MUP and coumarin 4 components of the mixture, respectively. Since the emission measurements were internally standardized by dividing them by the resorufin emission intensity $\left(E_{\lambda_r}\right)$, these equations become

$$\frac{E_{\lambda_M}}{E_{\lambda_r}} = \frac{E_{\lambda_M}^{(M)}}{E_{\lambda_r}} + \frac{E_{\lambda_M}^{(c)}}{E_{\lambda_r}} \quad (1)$$

$$\frac{E_{\lambda_c}}{E_{\lambda_r}} = \frac{E_{\lambda_c}^{(M)}}{E_{\lambda_r}} + \frac{E_{\lambda_c}^{(c)}}{E_{\lambda_r}} \quad (2)$$

The quantities of interest are the MUP emission at the MUP wavelength and the coumarin 4 emission at the coumarin 4 wavelength, $E_{\lambda_M}^{(M)}$ and $E_{\lambda_c}^{(c)}$, so equations (1) and (2) can be rearranged to

$$\frac{E_{\lambda_M}^{(M)}}{E_{\lambda_T}} = \frac{E_{\lambda_M}}{E_{\lambda_T}} - \frac{E_{\lambda_M}^{(c)}}{E_{\lambda_T}} \tag{3}$$

$$\frac{E_{\lambda_c}^{(c)}}{E_{\lambda_T}} = \frac{E_{\lambda_c}}{E_{\lambda_T}} - \frac{E_{\lambda_c}^{(M)}}{E_{\lambda_T}} \tag{4}$$

In other words, the emission by MUP at the MUP wavelength equals the total emission minus the emission by coumarin 4 at the MUP wavelength, and the emission by coumarin 4 at the coumarin 4 wavelength equals the total emission minus the emission by MUP at the coumarin 4 wavelength. By collecting the emission spectra of MUP and coumarin 4 standards, we can measure the ratio of shoulder-to-peak emission for both MUP and coumarin 4:

$$k^{(M)} = \frac{E_{\lambda_c}^{(M)}}{E_{\lambda_M}^{(M)}} \text{ and } k^{(c)} = \frac{E_{\lambda_M}^{(c)}}{E_{\lambda_c}^{(c)}}$$

The shoulder-to-peak ratios were found to be constant over the concentration ranges used in this study, so these ratios can be rearranged and substituted into equations (3) and (4) to give

$$\frac{E_{\lambda_M}^{(M)}}{E_{\lambda_T}} = \frac{E_{\lambda_M}}{E_{\lambda_T}} - k^{(c)} \frac{E_{\lambda_c}^{(c)}}{E_{\lambda_T}} \tag{5}$$

$$\frac{E_{\lambda_c}^{(c)}}{E_{\lambda_T}} = \frac{E_{\lambda_c}}{E_{\lambda_T}} - k^{(M)} \frac{E_{\lambda_M}^{(M)}}{E_{\lambda_T}} \tag{6}$$

Substitution of equation (5) into equation (6) followed by rearrangement gives

$$\frac{E_{\lambda_c}^{(c)}}{E_{\lambda_T}} = \frac{E_{\lambda_c}}{E_{\lambda_T}} - \frac{k^{(M)}}{\left(1 - k^{(M)}k^{(c)}\right)}\left(\frac{E_{\lambda_M}}{E_{\lambda_T}}\right) \tag{7}$$

The left-hand side of (7) is the internally standardized emission by only the coumarin 4 at the coumarin 4 emission wavelength (*i.e.*, the signal of interest). The right-hand side of (7) contains observed internally-standardized fluorescence emission at the coumarin 4 and MUP wavelengths and the measured shoulder-to-peak ratios for the two individual components of the mixture obtained by measurement of standards.