Using the dual-wavelength fluorophore LysoSensor Yellow/Blue for acidic-pH sensing at high hydrostatic pressures

HECTOR MICHAEL DE PEDRO, PAUL URAYAMA, Miami University — Spectroscopic techniques using dual-wavelength fluorophores are being developed for pH sensing under biologically-relevant high-pressure conditions (up to 1000 atm). Here we present the effects of pressure on the acid-base equilibrium of a commercially-available fluorophore used for sensing acidic pH—LysoSensor Yellow/Blue DND-160. Previous studies on the pressure response of near-neutral pH fluorophores including seminaphthofluoresceins and seminapthorhodafluors, show that the predominant effect is a pressure-dependent change in the equilibrium constant of the fluorophore’s acid-base reaction. That is, emission spectra from the protonated and deprotonated fluorophore forms did not show significant pressure sensitivity. For DND-160, the emission spectrum of the deprotonated form is highly pressure sensitive, likely due to effects on the rotational dynamics of the aromatic ring motif. Nonetheless, spectra remain interpretable in terms of its associated acid-base reaction, indicating that DND-160 is still useful for high-pressure, low-pH sensing.

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Paul Urayama
Miami University

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