

Dual-Stimuli-Responsive Small Molecular Probes for Quantitative Detection of Both pH and Enzyme Activity

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Keywords: Photoluminescence; Dual-Responsive Probe; Bipyridyl; Quantification

Determination and quantification of multiple analytes are important for better understanding those physiological functions which involve the coincidence of multiple chemical events. However, very few probes that respond to multiple stimuli, especially to an enzyme, have been reported. In this contest, we have developed the first dual-responsive probe for quantitative detection of pH as well as enzyme activity in an unknown sample. 3,3'-Dihydroxy-2,2'-bipyridyl derivative **3** whose molecular weight is less than 200 is easy to be functionalized. Because two sterically demanding substituents at oxygen functionality of bipyridine induced its twisted structure, probe **1** is non-emissive. However, the removal of a

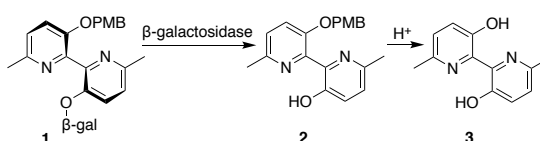


Figure 1. Activation process of probe **1**.

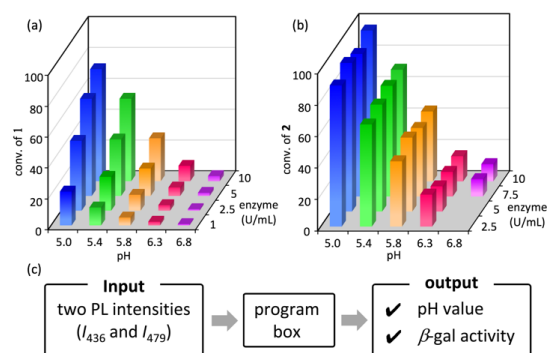


Figure 2. (a) and (b): Three-dimensional graphs of the conversion of **1** and **2** with pH values and enzyme activities. (c) Flow chart of the calculation program.

β -galactosyl (β -gal) moiety followed by the deprotection of *p*-methoxybenzyl (PMB) group gave emissive **3** through intermediate **2** whose emission is different from **3** (Figure 1). Moreover, probe **1** could emit strong photoluminescent signals in ovarian cancer cells OVK-18, while it emitted negligible photoluminescence in normal human cells HUVEC. Three-dimensional graphs of the conversion of **1** and **2** with pH values and β -galactosidase's activities (Figure 2a and 2b) allowed us to build up a calculation program (Figure 2c). Inputting the photoluminescence (PL) intensity at 436 nm and 479 nm into the program box automatically produces outputs of calculated pH value and enzyme activity with high accuracy. Relative errors of the pH and the β -galactosidase activity between the calculated value and the experimental value were below $0.6\% \pm 0.1\%$ and $7\% \pm 2\%$, respectively. Therefore, this dual-responsive probe will earn a place in bioimaging area for monitoring multiple analytes with high efficiency.