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# Coumarin-based, switchable fluorescent substrates for enzymatic bacterial detection

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## Abstract

Enzymatically-switchable fluorescent substrates, such as the commercially available 4-methyl umbelliferones (4-MU) are used as standard indicators of enzymatic activity for the detection of various microorganisms and pathogens. However, a major disadvantage of 4-MU is its relatively high pKa leading to only partial dissociation of the fluorescent anion under the conditions where the enzymes are most effective (pH 6-6.5). Here we present a method for new, enzymatically-switchable, fluorescent substrates with improved photo-physico/chemical properties. The lead derivative, 4-AAU, shows excellent solubility in aqueous media (0.81mg/mL) when compared to 4-MU (0.16mg/mL), significantly improved quantum yield and wider dynamic range of its fluorescence properties. The corresponding bacterial substrate  $\beta$ -4-AAUG showed superior selectivity in the detection of clinically relevant amounts of *E. coli*, *Enterococcus* and *K. pneumonia* (1 CFU). The fluorescence intensity of  $\beta$ -4-AAUG was almost 5 times higher than that of the standard, the detection was possible in reasonably short time (~2.5 hours) and with excellent sensitivity.

## Graphical Abstract

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