A FLUORESCENT HYDROGEL-BASED FLOW CYTOMETRY SCREENING PLATFORM FOR HYDROLYTIC ENZYMES

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In directed evolution experiments and enzyme discovery, screening throughput plays a key role. In this study, for the first time a high-throughput screening platform based on a coupled reaction of glucose oxidase and a hydrolase (*Yersinia mollaretii* phytase [YmPh]) is described. The coupled reaction produces hydroxyl radicals through Fenton's reaction, which initiate a poly(ethylene- glycol)-acrylate-based polymerization incorporating a fluorescent monomer. Consequently, a fluorescent hydrogel is formed around *Escherichia coli* cells expressing active YmPh. We validate the performance of the fluorescent polymer shell (fur-shell) technology by directed phytase evolution that yielded variant M1 with 97 U/mg increased specific activity compared to YmPh wild type (315 U/mg). Thus, fur-shell technology represents a rapid and nonlaborious way of identifying the most active variants from vast populations, as well as a platform for generation of polymer-hybrid cells for biobased interactive materials.

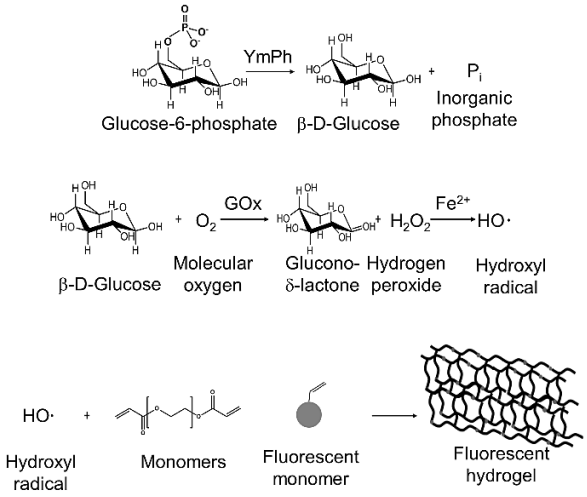

Figure 1 – Coupled YmPh/glucose oxidase (GOx) reaction leads to a fluorescent hydrogel shell formation

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