

Enzyme Cascade Immobilization with Porous Coordination Cages: Application to Paper-based Cyanide Detection

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Keywords: *Enzyme immobilization, Enzymatic cascade, Coordination cage, Metal-organic polyhedra, Paper-based sensors*

Enzyme immobilization is an essential technological development for biocatalysis, as it greatly improves the recyclability and stability of usually hard to isolate and sensitive enzymes for numerous applications. Recently, our research group established water-soluble porous coordination cages (PCCs) as a versatile platform for enzyme bio-immobilization. The PCCs enabled the easy immobilization of enzymes with high retention of catalytic performance in water, by forming charge-driven PCC-enzyme aggregate.¹ Since then, the method has been expanded to the co-immobilization of enzymes with additional filler proteins, enabling systematic and easy tuning of enzyme concentration in the aggregates and greatly reducing limitations related to mass transfer.²

In this presentation, we describe the systematic co-assembly of several different enzymes with PCCs to establish cascade reactions (Figure 1). We notably explored the catalytic couple of glucose oxidase (GOx) and horseradish peroxidase (HRP) as a model system, enabling the colorimetric detection of glucose through the oxidation of a chromogenic dye. The development of efficient system is greatly assisted by our reliable and near quantitative immobilization methods, which allows the easy tuning of enzyme ratio for an ideal cascade throughput.

Another benefit of the immobilization method is its bottom-up self-assembly nature, forming micrometer-sized aggregates from the association of nanometer-sized entities. Taking advantage of this, we developed a strategy to reliably immobilize enzymes in the standardized porosity of cellulose filter-paper, with excellent retention of enzyme content. Combining all these, we used the method to develop paper-based sensors for naked-eye detection of analytes. In particular, using the strongly inhibiting effect of cyanides anions on peroxidase activity, a new type of cyanide detection system was successfully developed.

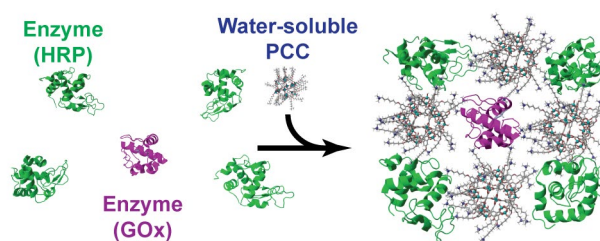


Figure 1. Immobilization of the GOx-HRP enzyme cascades with water-soluble PCCs.

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