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A High-Throughput Enzyme Screening Method Using Broadband Coherent Anti-stokes Raman Spectroscopy

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Abstract : Enzymes have attracted increasing attentions in industrial manufacturing for their applicability in catalyzing complex chemical reactions under mild conditions. Directed evolution has become a powerful approach to optimize enzymes and exploit their full potentials under the circumstance of insufficient structure-function knowledge. With the incorporation of cell-free synthetic biotechnology, rapid enzyme synthesis can be realized because no cloning procedure such as transfection is needed. Its open environment also enables direct enzyme measurement. These properties of cell-free biotechnology lead to excellent throughput of enzymes generation. However, the capabilities of current screening methods have limitations. Fluorescence-based assay needs applicable fluorescent label, and the reliability of acquired enzymatic activity is influenced by fluorescent label's binding affinity and photostability. To acquire the natural activity of an enzyme, another method is to combine pre-screening step and high-performance liquid chromatography (HPLC) measurement. But its throughput is limited by necessary time investment. Hundreds of variants are selected from libraries, and their enzymatic activities are then identified one by one by HPLC. The turn-around-time is 30 minutes for one sample by HPLC, which limits the acquirable enzyme improvement within reasonable time. To achieve the real high-throughput enzyme screening, i.e., obtain reliable enzyme improvement within reasonable time, a widely applicable high-throughput measurement of enzymatic reactions is highly demanded. Here, a high-throughput screening method using broadband coherent anti-Stokes Raman spectroscopy (CARS) was proposed. CARS is one of coherent Raman spectroscopy, which can identify label-free chemical components specifically from their inherent molecular vibration. These characteristic vibrational signals are generated from different vibrational modes of chemical bonds. With the broadband CARS, chemicals in one sample can be identified from their signals in one broadband CARS spectrum. Moreover, it can magnify the signal levels to several orders of magnitude greater than spontaneous Raman systems, and therefore has the potential to evaluate chemical's concentration rapidly. As a demonstration of screening with CARS, alcohol dehydrogenase, which converts ethanol and nicotinamide adenine dinucleotide oxidized form (NAD+) to acetaldehyde and nicotinamide adenine dinucleotide reduced form (NADH), was used. The signal of NADH at 1660 cm^{-1}, which is generated from nicotinamide in NADH, was utilized to measure the concentration of it. The evaluation time for CARS signal of NADH was determined to be as short as 0.33 seconds while having a system sensitivity of 2.5 mM. The time course of alcohol dehydrogenase reaction was successfully measured from increasing signal intensity of NADH. This measurement result of CARS was consistent with the result of a conventional method, UV-Vis. CARS is expected to have application in high-throughput enzyme screening and realize more reliable enzyme improvement within reasonable time.

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