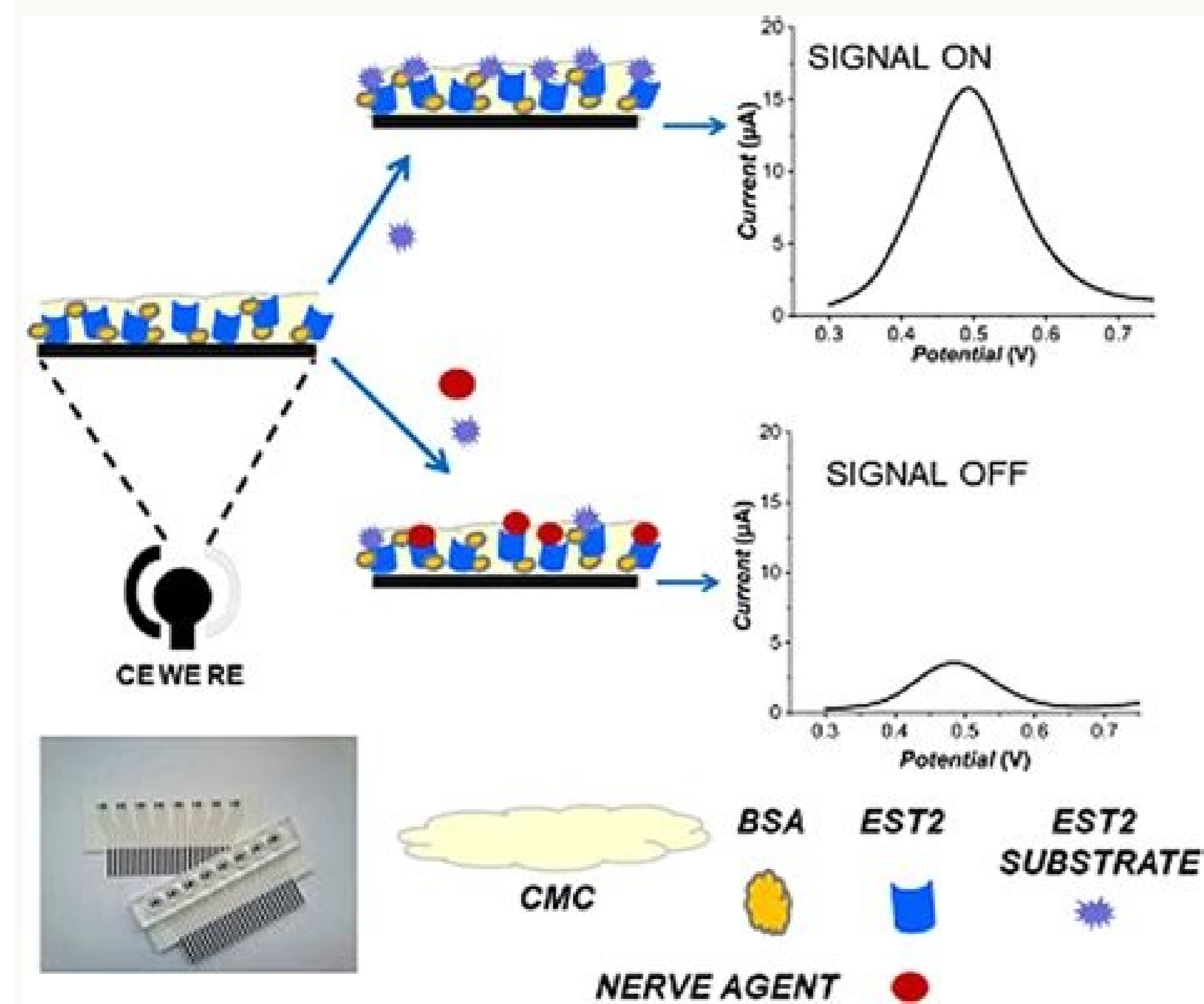
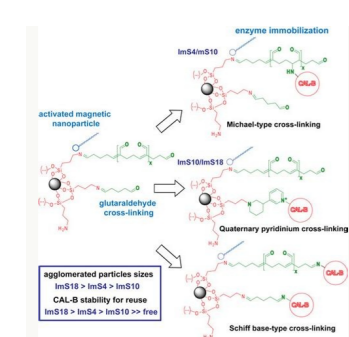


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Regeneration of Cofactors for Enzyme Biocatalysis

5

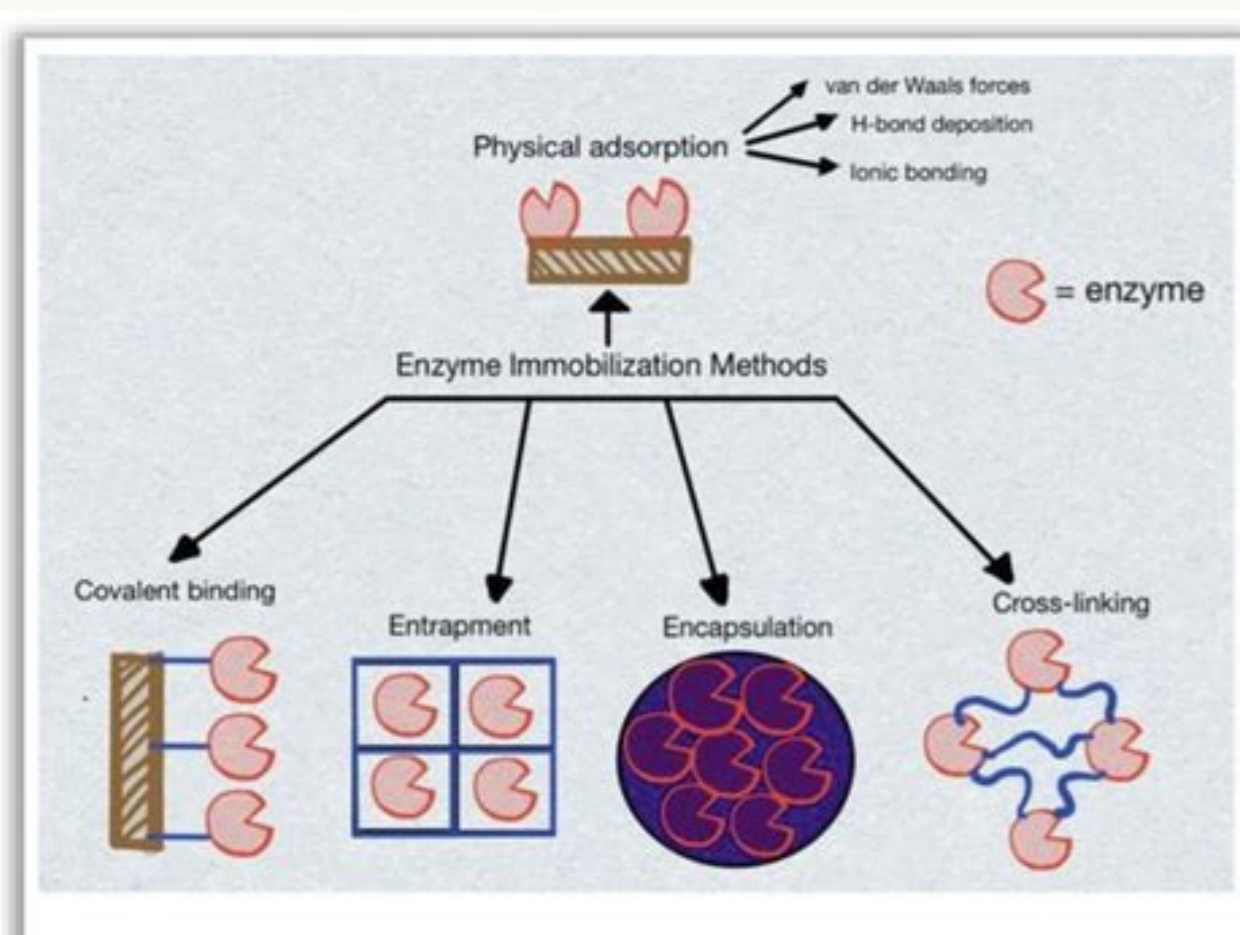
Ryan D. Woodyer, Tyler W. Johannes and Huimin Zhao

1. INTRODUCTION

Industrial biocatalytic processes continue to become increasingly common as interest in the area multiplies (Faber 2000, Koeller & Wong 2001, Schmid et al. 2001, Schoemaker et al. 2003). This interest is driven by recent advances in genomics and genetic engineering coupled with the need for more selective, safer, and cleaner reactions. Enzymes as catalysts meet many of the needs of industrial processes. However, most of the biocatalysts in current use are limited to cofactor-independent enzymes such as hydrolases, which perform relatively simple chemistry (Faber 2000). In contrast, cofactor-dependent enzymes, such as oxidoreductases and transferases, can perform more complex chemistry. Many of these reactions are synthetically useful. For example, nicotinamide adenine dinucleotide/(NAD)dependent oxidoreductases catalyze the asymmetric reduction of carbonyl groups to alcohols and amines (Stewart 2001, Li et al. 2002) and acetyl-Coenzyme A-dependent (AcCoA) synthetases catalyze asymmetric carbon-carbon bond formation (Patel et al. 1986, Ouyang et al. 1990, Jossek & Steinbuchel 1998, Satoh et al. 2003). However, because these enzymes use cofactors, they have seen little use in large-scale applications.

Cofactors such as NAD and AcCoA are low molecular weight compounds that are essential for many enzymatic reactions. Some cofactors such as adenosylcobalamin, pyridoxal phosphate, biotin, and flavins are tightly bound to the enzymes and are self-regenerating in most cases. However, other cofactors such as pyridine dinucleotides (NAD(P)(H)) and nucleoside triphosphates (NTPs) act more like cosubstrates. They are loosely bound ($K_D \sim \mu\text{M}-\text{mM}$) and act as functional group transfer agents, and therefore are consumed in stoichiometric amounts (Chenault et al. 1988). The limited use of enzyme-cofactor reactions in industry stems from the high cost of cofactors, which ranges from \$30/mmol for NAD, to several orders of magnitude more expensive for cofactors such as AcCoA and 3-phosphoadenosine-5'-phosphosulfate (PAPS). This high cost prohibits stoichiometric addition of cofactors, so they must be regenerated *in situ* for any large-scale reaction. Cofactor regeneration can also drive the reaction to completion, simplify product isolation, and allow the removal of inhibitory cofactor by-products, further reducing the cost of synthesis (Chenault et al. 1988). Various methodologies of cofactor regeneration have been developed to allow the use of catalytic amounts of cofactors (Chenault & Whitesides 1987, Chenault et al. 1988, Wong & Whitesides 1994, Koeller & Wong 2001, van der Donk & Zhao 2003, Zhao & van der Donk 2003) and many are described in this chapter.

In order for cofactor regeneration to be economical and practical, several requirements must be met. First and foremost, the total turnover number (TTN) or the total number of moles of product formed per mole of cofactor during the course of a complete reaction must be high (Chenault & Whitesides



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