

## Electroenzymatic synthesis with H<sub>2</sub>O<sub>2</sub>-dependent enzymes

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### Motivation

Heme-containing **Peroxidases/Peroxygenases**

- + Transfer oxygen to various substrates
- + Can catalyze **similar reactions as P450** monooxygenases
- + **Don't need costly cofactors** like NAD(P)H (as P450s)
- Use H<sub>2</sub>O<sub>2</sub> as co-substrate
- Are irreversibly inactivated by **excess H<sub>2</sub>O<sub>2</sub>**

**Example:**

Chloroperoxidase (CPO) from *Caldariomyces fumago*

Phosphate buffer, 50 μM H<sub>2</sub>O<sub>2</sub> → **Half-life time: 38 min<sup>1</sup>**

There is need for the development of an **easy to set up, energy-efficient** technique to **prevent peroxidase and peroxygenase inactivation** by excess hydrogen peroxide and enable **high total turnover numbers (ttn)**.

### Strategy

Electrochemical **in situ** production of H<sub>2</sub>O<sub>2</sub>

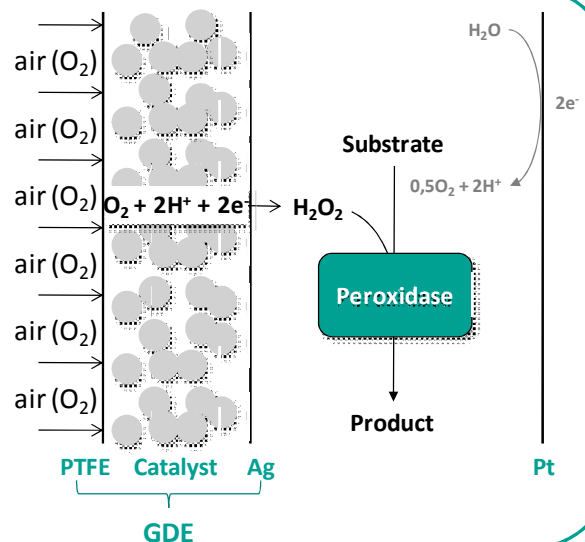
- + **In situ** production **evades volume increase** during long run-times
- + **No disturbing side-products** are produced
- + **Atom-efficient** H<sub>2</sub>O<sub>2</sub>-production

Use of a **gas diffusion electrode (GDE)**

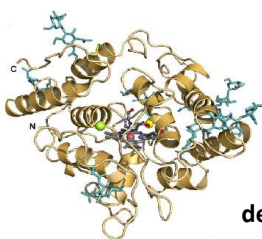
- + Reduces problems related to **oxygen solubility and diffusibility**
- + **No need for costly aeration**

Application on various **H<sub>2</sub>O<sub>2</sub>-dependent but -sensitive enzymes**

- Chloroperoxidase from *Caldariomyces fumago*
- **Unspecific peroxygenase from *Agrocybe aegerita***
- P450 monooxygenase OleT<sub>JE</sub> from *Jeotgalicoccus spec*



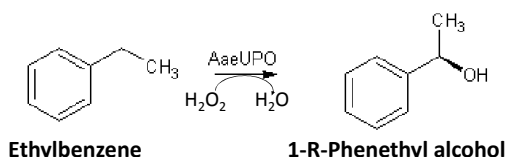
### Results



**Unspecific peroxygenase** from *Agrocybe aegerita* (**AaeUPO**) is a **heme-thiolate peroxygenase**.

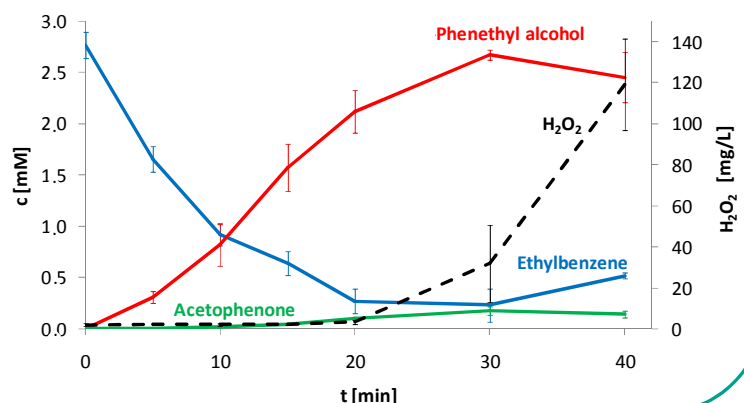
It catalyzes **peroxide dependent oxygenations** of diverse substrates.

**Model reaction:**



First tests in a **non-optimized GDE-based** system reached a **ttn of 20,070** (97% conversion).

The **highest published ttn** for this model reaction is **43,000** by feeding a **dilute H<sub>2</sub>O<sub>2</sub>-solution**.<sup>2</sup>



[1] Seelbach, K. 1997 – Dissertation [2] Kluge, M. et al., 2012 - 14,440 Green Chemistry