

## ENZYMES IN AMPHIPHILIC CONETWORKS

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**INTRODUCTION:** The technological and synthetical utility of enzymes can be enhanced greatly by using them in organic solvent rather than their natural aqueous reaction media [1]. However, drawbacks of enzymes in organic media are their insolubility resulting in lower catalytic activity and their reduced stability. We have developed a method to significantly reduce these problems: incorporating an enzyme into the hydrophilic phase of an amphiphilic conetwork.

**METHODS:** Amphiphilic conetworks on the basis of polyhydroxyethylacrylate (PHEA) and polydimethylsiloxan (PDMS) were synthesized by radical copolymerisation of  $\alpha,\omega$ -methacryloyl-terminated PDMS-macromonomers and trimethylsilyl-protected HEA and subsequent cleavage of the protecting group [2]. The networks were produced as thin films covalently attached to a glass substrate. These films were incubated in an enzyme containing aqueous solution and thus loaded with the biocatalyst. After drying, the enzyme loaded networks were placed in organic media, allowing the enzyme to catalyze a reaction of organosoluble substrates.

Enzymatic activity of horseradish peroxidase (HRP) was studied through the oxidative coupling of phenol and N,N-dimethylphenyldiamine with *tert*-butylhydroperoxide in n-heptane. The reaction was carried out directly in a cuvette and was monitored by the increase of absorbance at  $\lambda = 536$  nm.

**RESULTS:** Amphiphilic conetworks were synthesized as thin films to minimize diffusion problems in enzymatic reactions and to allow their use as catalytic coatings. The films show nanophase separation on the surface and in their bulk (Fig.1) and can be swollen in water as well as in organic solvents.

By loading the film with HRP, this biocatalyst was entrapped into the 10-100 nanometer sized domains of the network's hydrophilic phase. This phase acts as a nanoreactor by providing enzyme compatible surroundings and simultaneously allowing good accessibility for organosoluble substrates through a swollen hydrophobic phase.

HRP was used as model enzyme to catalyze a reaction in heptane. It showed an up to 100-fold increased catalytic activity as well as increased stability when entrapped into the amphiphilic film, compared to native HRP directly suspended into the reaction mixture (Fig. 2).

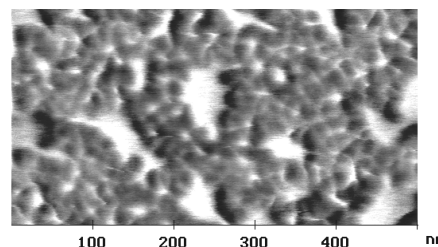


Fig. 1: Nanophase separation on the surface of a thin amphiphilic conetwork film (PHEA-I-PDMS, 50 % PHEA) revealed by phase contrast AFM. PDMS shows dark, PHEA light.

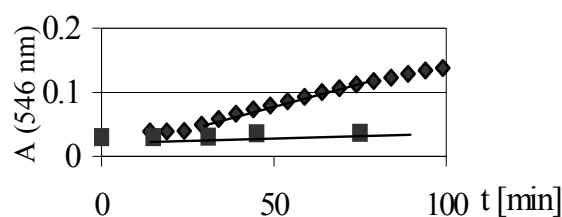


Fig. 2: Increased catalytic activity of HRP entrapped in an amphiphilic conetwork (◆) compared to native HRP (■): HRP-catalysed oxidative coupling of phenol and N,N-dimethylphenyldiamine with *tert*-butylhydroperoxide in n-heptane monitored by the increase of absorbance at  $\lambda = 536$  nm.

**DISCUSSION & CONCLUSIONS:** For the first time it could be shown that nanophase separated amphiphilic conetworks possess the ability to stabilize and enhance the catalytic activity of enzymes in organic solvents dramatically. This could lead to new biocatalytic systems (e.g. catalytic coatings for reactors) as well as extend the application of biocatalysts in organic synthesis.

**REFERENCES:** <sup>1</sup>A.M. Klivanov (2001) *Nature* **409**:241-46. <sup>2</sup>J. Scherble, B. Ivan, R. Mülhaupt (2002) *Macromol Chem Phys* **203**, 12:1866-71.

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