

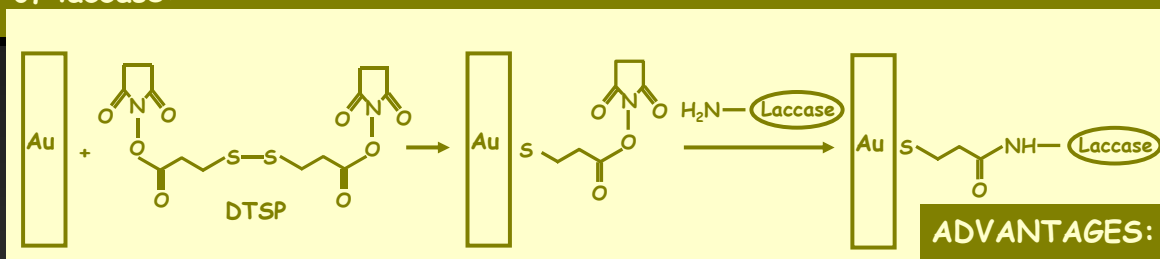
DETERMINATION OF PHENOLIC COMPOUNDS WITH AN AMPEROMETRIC LACCASE BIOSENSOR BASED ON A GOLD ELECTRODE MODIFIED WITH A SELF-ASSEMBLED MONOLAYER



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1. Exposure of AuE to solutions of DTSP gives rise to the modification of the surface with N-succinimidyl-3-propionate (NTSP) which can react with aminogroups allowing for the covalent immobilisation of laccase:

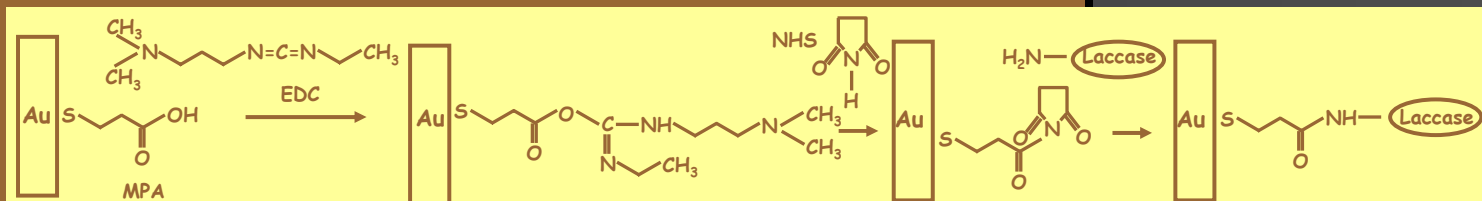


ADVANTAGES:

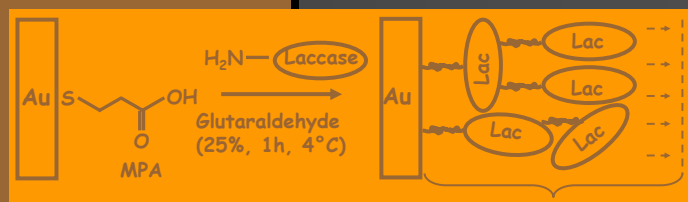
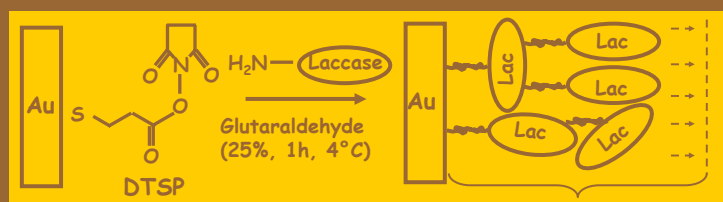
- Short time of formation of the monolayer (1h)
- Fast cleaning of the AuE
- No derivatisation needed

2. This covalent immobilisation procedure was compared with:

(i) a covalent binding using carbodiimide chemistry, on a self-assembled monolayer of 3-mercaptopropionic acid (MPA) deposited on the AuE (which is the traditional method used in our laboratory), and

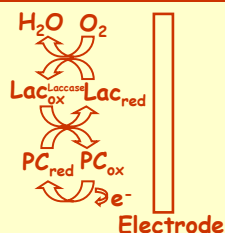


(ii) a cross-linking with glutaraldehyde.



3. The behaviour of the different biosensors was evaluated in terms of sensitivity and operational range for caffeic acid ($E_{app} = 0.0$ V; Citrate buffer 0.1M, pH 5.0):

REACTION SCHEME:



BIOSENSOR	SLOPE, A/M	LINEAR RANGE, M	DL, M
AuE-DTSP-Lac (cov)	$8.34 \times 10^{-4} \pm 6.86 \times 10^{-5}$	$2.0 \times 10^{-6} - 1.0 \times 10^{-5}$	1.3×10^{-6}
AuE-MPA-Lac (cov)	$7.58 \times 10^{-4} \pm 3.22 \times 10^{-5}$	$2.0 \times 10^{-6} - 1.0 \times 10^{-5}$	1.2×10^{-6}
AuE-DTSP-Lac-Glut	$3.34 \times 10^{-2} \pm 1.49 \times 10^{-3}$	$1.0 \times 10^{-7} - 1.0 \times 10^{-5}$	7.2×10^{-8}
AuE-MPA-Lac-Glut	$2.98 \times 10^{-2} \pm 2.34 \times 10^{-3}$	$2.0 \times 10^{-7} - 1.0 \times 10^{-5}$	8.8×10^{-8}

4. The AuE-DTSP-Lac-Glut biosensor was used to detect a large number of phenols (3-Hydroxybenzoic acid, 4-Hydroxybenzoic acid, 4-Hydroxyphenylacetic acid, catechol, 3,4-Dihydroxyphenylacetic acid, 3,4-Dihydroxyphenylpropionic acid and caffeic acid) occurring in olive oil mill wastewater. Preliminary studies showed promising results.