The inhibition study of cytochrome *bd* oxidase using the enzyme-based electrochemical sensor

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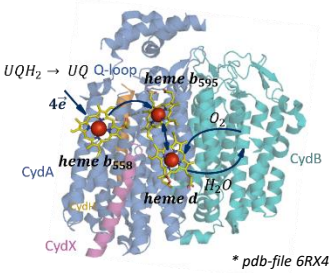
INTRODUCTION

The cytochrome *bd* oxidases, membrane enzymes found only in the respiratory chain of the prokaryotes, are of large interest for the development of future antibiotics as they are believed to be involved in bacterial adaptability mechanisms. They catalyze the reduction of molecular oxygen in water and oxidation of quinols and contribute to the proton motive force required for the ATP synthesis [1]. Due to their hydrophobic nature, membrane proteins are more difficult to handle than soluble proteins, thus method of protein film voltammetry has been applied.

Here, we have developed a biosensor for the study of terminal oxidases based on their immobilization on gold nanoparticles modified with a self-assembled monolayer of thiols [2,3]. This electrochemical sensor was used for the inhibition screening of a target-focused library of 34 compounds which belong to the families of quinones, naphthoquinones, phenols, quinolones, coumarins and flavonoids against cytochrome *bd* oxidase [4].

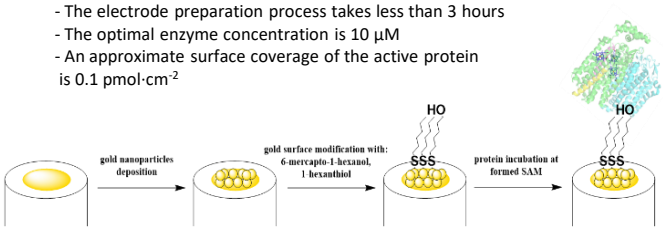


* pdb-file 6RX4

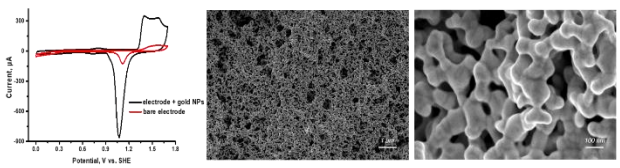
METHODS

PFV immobilization

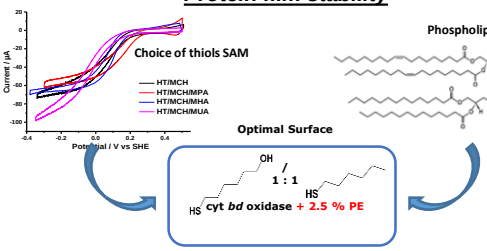
- The electrode preparation process takes less than 3 hours
- The optimal enzyme concentration is 10 µM
- An approximate surface coverage of the active protein is 0.1 pmol·cm^{-2}



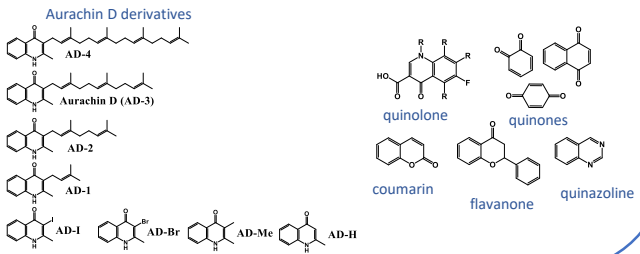
SEM and CV of an electrode modified with gold nanoparticles



Protein film stability

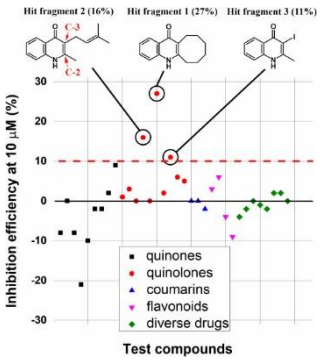


Target-focused Compound Library



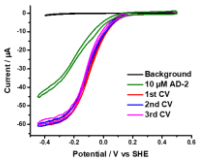
RESULTS

Primary Inhibition Screening



- The quinolones with alkyl and iodine substituents in C-2 and C-3 were identified as leads out of 34 molecules library
- The quinolones with no substituent or polar substituents such as hydroxy, carboxylic acids or esters showed no or little inhibition towards cyt *bd* oxidase

Aurachin D derivatives inhibition activity

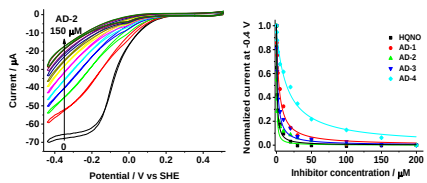


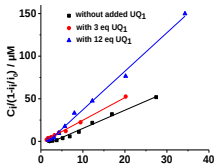
AD-4 n=4 ⟨ AD-3 n=3 ⟨ AD-2 n=2 ≈ HQNO ⟩ AD-1 n=1

Compound	$(di/i)_L - (di/i)_0$, % without UQ	$(di/i)_L - (di/i)_0$, % 1 cyt bd : 3 UQ	$(di/i)_L - (di/i)_0$, % 1 cyt bd : 12 UQ
HQNO	21 ± 3.1	24 ± 3.4	27 ± 4.4
AD-1	16 ± 1.1	17 ± 2.4	19 ± 1.1
AD-2	37 ± 1.8	23 ± 3.8	15 ± 0.7
AD-3	27 ± 1.2	19 ± 3.9	10 ± 0.1
AD-4	6 ± 2.3	7 ± 1.5	-
AD-I	11 ± 0.4	6 ± 0.6	-
AD-Br	6 ± 0.9	10 ± 4.4	-
AD-Me	5 ± 3.1	2 ± 1.3	-
AD-H	1 ± 0.2	3 ± 0.6	-

- The length of isoprenoid chain plays crucial role on inhibition in synthesized derivatives of Aurachin D
- Inhibition effectiveness decreases as follows: AD-2 > AD-3 ≈ HQNO > AD-1 > AD-4 ≈ AD-I ≈ AD-Br ≈ AD-Me > AD-H

Half-inhibitory Concentration determination





Compound	IC_{50}, µM
HQNO	1.1 ± 0.2
AD-1	3.8 ± 0.1
AD-2	1.1 ± 0.7
AD-3	2.2 ± 0.5
AD-4	14.4 ± 0.5

- AD-2 is the most active inhibitor against *bd* oxidase, not natural Aurachin D as was reported before in literature
- The results point on the competitive binding of the AD-2 and AD-3 with the natural substrate UQ

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