Design of an electrochemical assay for identification of cytochrome *bd* oxidases inhibitors

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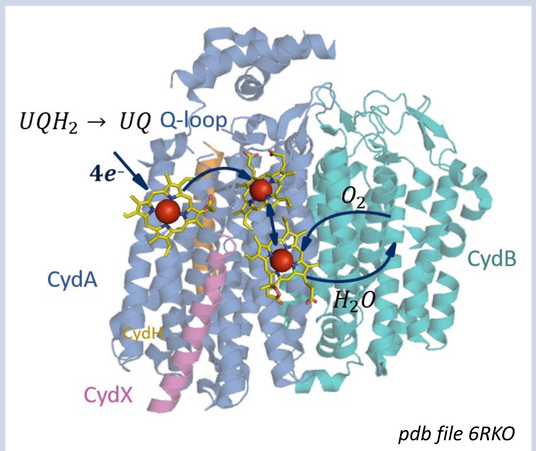

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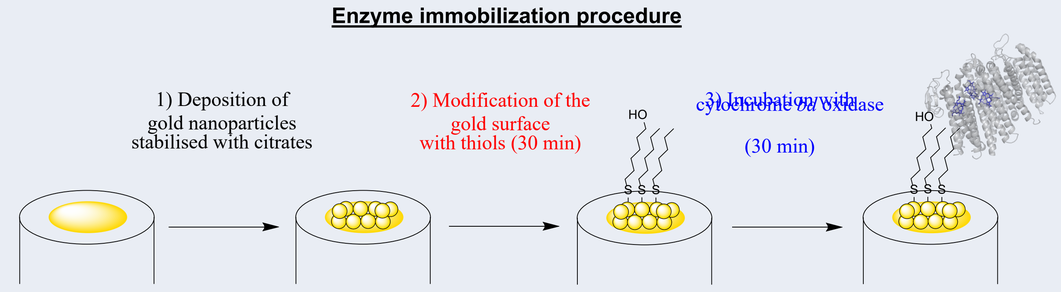
Introduction

Cytochrome *bd* oxidases are bacterial only membrane proteins which catalyze the reduction of O_2 by two equivalents of quinol and contribute to the proton motive force required for ATP synthesis by taking the four protons required for O_2 reduction from the cytoplasmic side and releasing protons from quinol oxidation to the periplasmic side of the membrane [1]. They also play a crucial role in the bacterial virulence [2], the tolerance to oxidative and nitrosative stress conditions [3] and the resistance to antibiotics [4], although the molecular mechanisms are not fully understood. Specific inhibitors of these proteins may thus find immediate applications as antibacterial drugs with new mode of action.

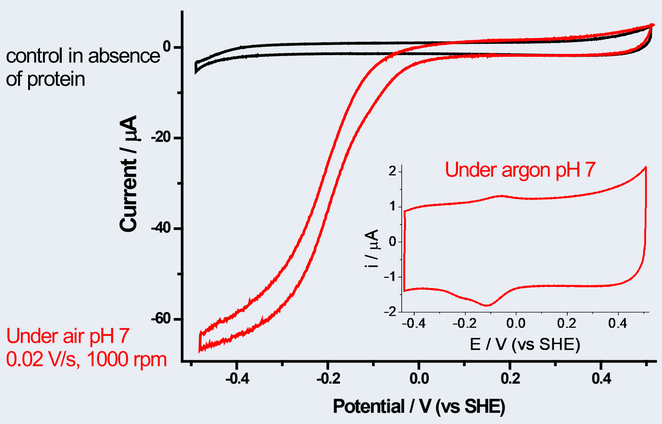
To study the oxygen reductase activity and inhibition of cytochrome *bd* oxidases, we have developed an electrochemical assay based on the immobilization of the enzymes on 3D gold nanoparticle electrodes modified with self-assembled monolayers of thiols [5-6]. The gold nanoparticles are used to facilitate the electron transfer from the electrode to the enzyme cofactors located deep inside the enzyme.

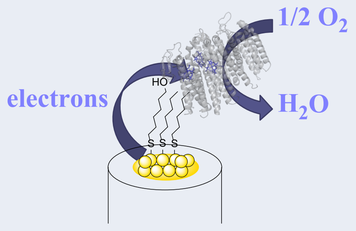
Assay development and optimization

Enzyme immobilization procedure

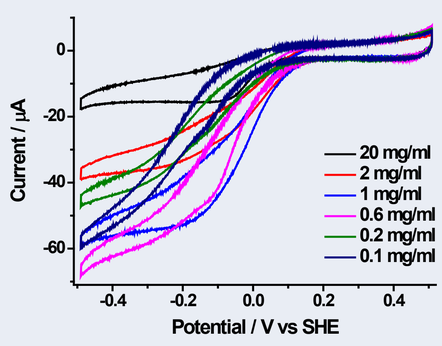


Protein film voltammetry

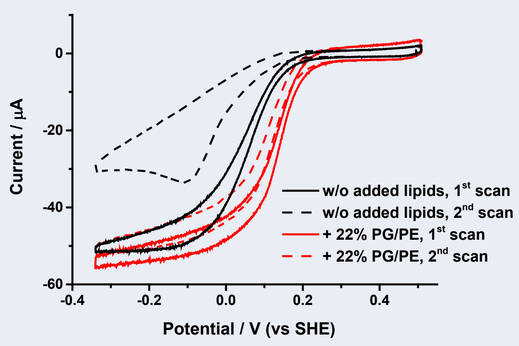




Influence of enzyme concentration

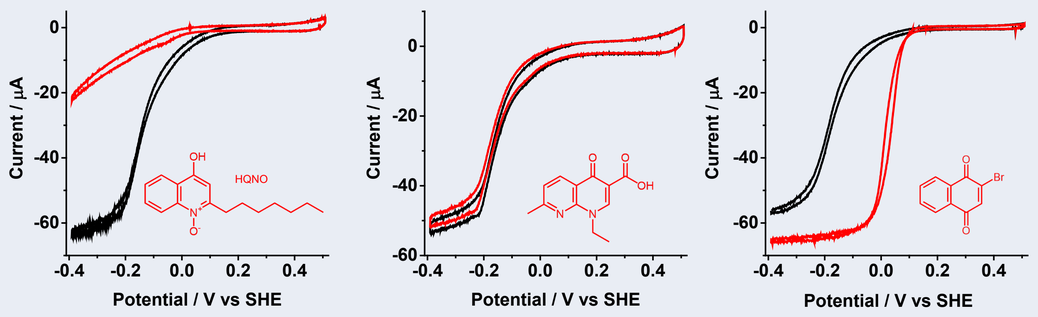


Influence of phospholipids addition

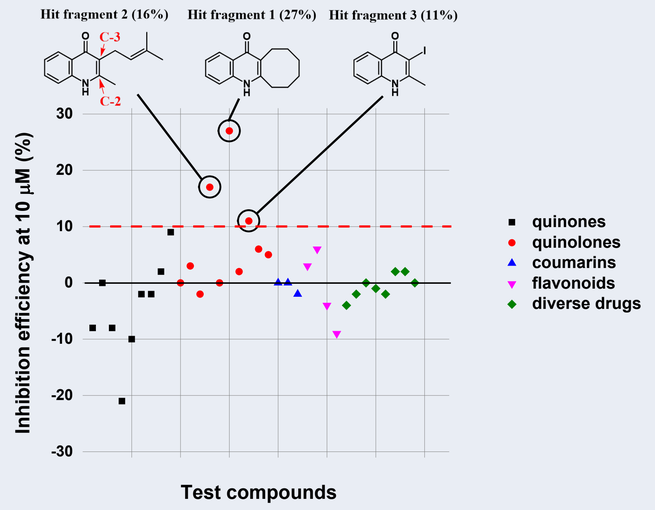


Screening of inhibitors

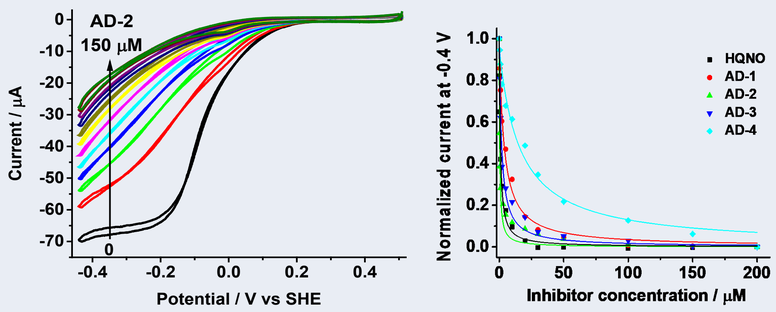
Effect of the addition of quinone-like molecules on the voltammograms of cytochrome *bd* oxidase

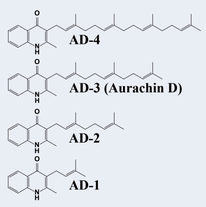


Primary screening campaign



Focus on modified aurachin D derivatives





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

Conclusion

The direct electrochemical characterization of cytochrome *bd* oxidase from *E. coli* was achieved. The activity is lower for densely packed layers of proteins and the stability of the protein films is improved by the addition of lipids.

Protein film voltammetry also allowed to detect the inhibition of these key redox proteins by small quinol analogs [7]. Quinolones with alkyl or iodine substituents in position C-2 and C-3 were identified as potential inhibitors of the enzyme from a a focused library of 35 compounds related to the quinol substrate of the enzyme, including quinones, naphthoquinones, phenols, quinolones, coumarins and flavonoids. More potent inhibitors were obtained by chemical modification of the quinolone core and introduction of an isoprenyl chain in position C-3. The activity of these competitive inhibitors increases from one to two isoprene repetitive units and decreases for longer chains.

References

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Acknowledgements

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