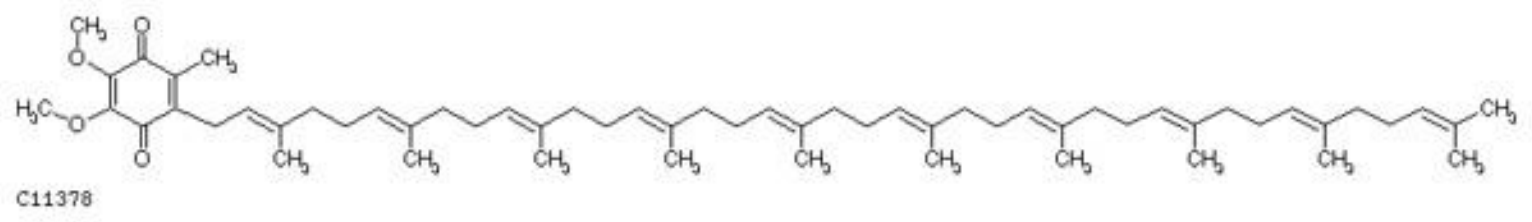
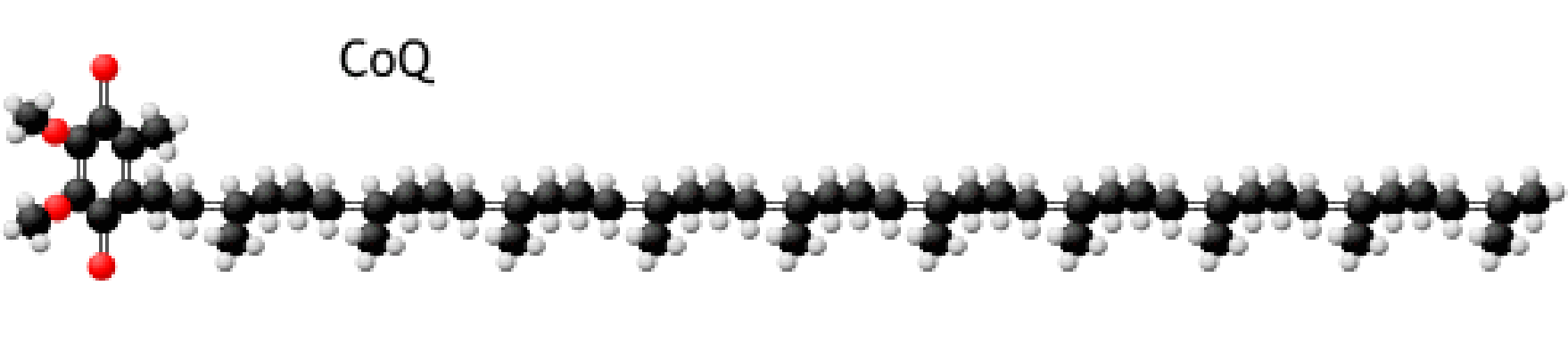
NEW ASPECTS INTO THE CHEMISTRY OF COENZYME Q10 FAMILY MEMBERS
(calcium binding and transporting)



Rubin Gulaboski, Ivan Bogeski,
Valentin Mirceski, Reinhard Kappl,
Markus Hoth



Rubin Gulaboski, Ivan Bogeski, Reinhard Kappl, Markus Hoth, ***„Benzoquinones based Antioxidants"***, European Patent Office, Munich 2010, **PATENT No. 09178735.8**.

STATE OF THE ART

Most quinones, with coenzyme Q10 as their best known representative, are seen as very efficient radical scavengers and antioxidants, commonly acting in a way to protect the living cells from oxidative damage. Due to their anti-oxidizing activity many quinones are used as therapeutic agents e. g. in oncology.

Coenzyme Q10 is widely used as a dietary supplement and in a number of cosmetics. Although its anti-oxidative effect is undisputable, Q10 has one big disadvantage: due to the long, hydrophobic side-chain it is only slightly water-soluble. The intestinal absorption is assumed to be less than 10%. So improvement of the aqueous solubility is a major concern. Besides the reduction of particle size another way to improve the solubility is the complexation in water with cyclodextrins. But also here the water-solubility is limited.

THE IDEA

The idea was to develop antioxidants based on the commonly known ubichinons but with an improved water-solubility.

This was achieved by an easy reaction out of the natural substances coenzyme Q0 to Q10. The ubichinons are transformed in polar molecules. The new substances show high solubility.

The aqueous solutions of the new compounds are also long-time stable, considerably more then less concentrated natural coenzyme Q-solutions.

Initial tests showed the high potential of the new compounds as antioxidants:

- Compared to the natural compounds the new once show a higher negative redox-potential
- Analyzing of reactions with oxygen showed that the new substances reacted much faster than the natural once
- The new compounds are way more insensitive to changes in pH. The redox-potential is not influenced in a range from pH 4 to 13.

APPLICATION AND BENEFITS

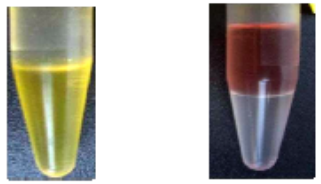
The use of the new synthesized coenzymes Q0-10 is possible same areas as the natural substances are used:

Cosmetics (skin care products, etc.), dietary supplements (e.g. against hypertension, migraine headaches, etc.), therapeutic agents

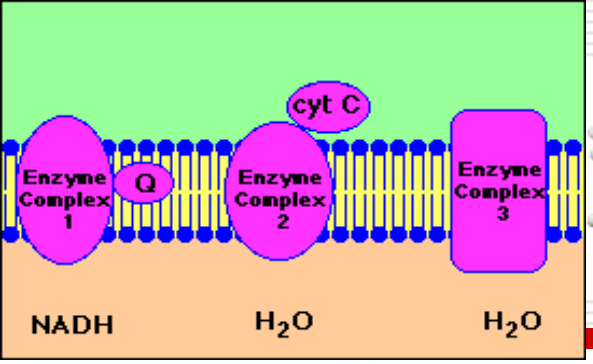
The new advantages of

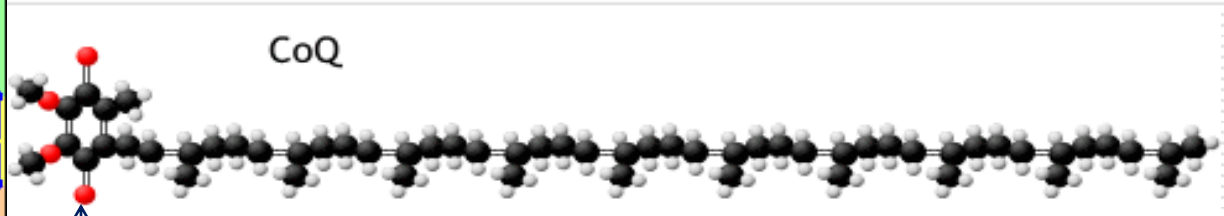
- better solubility
- higher stability
- stronger redox-potential and
- less sensitive to changes in pH,

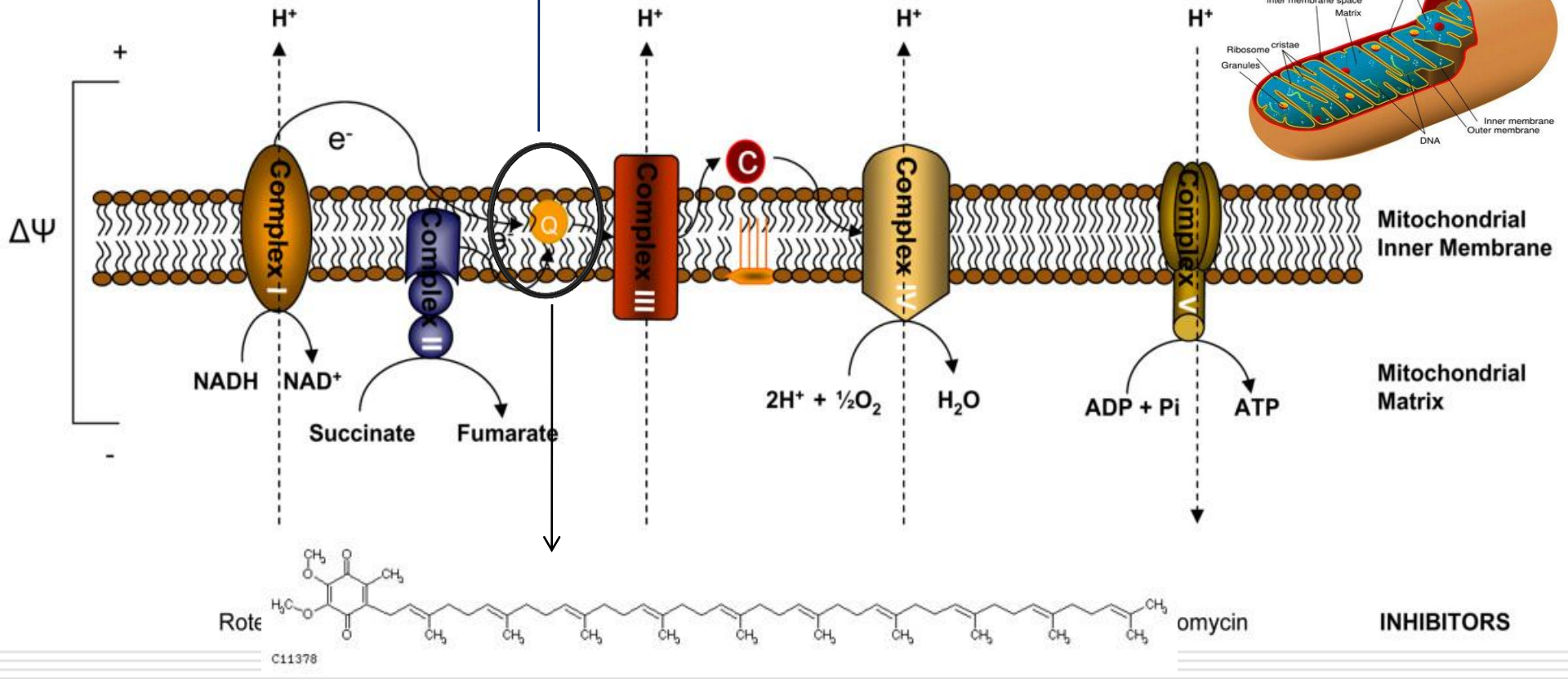
also new application areas which so far were not possible come into reach, since the so far low intestinal absorption of about 10% in humans would no longer be a hindrance. The new substances might even prove to be candidates for so far unknown application areas.



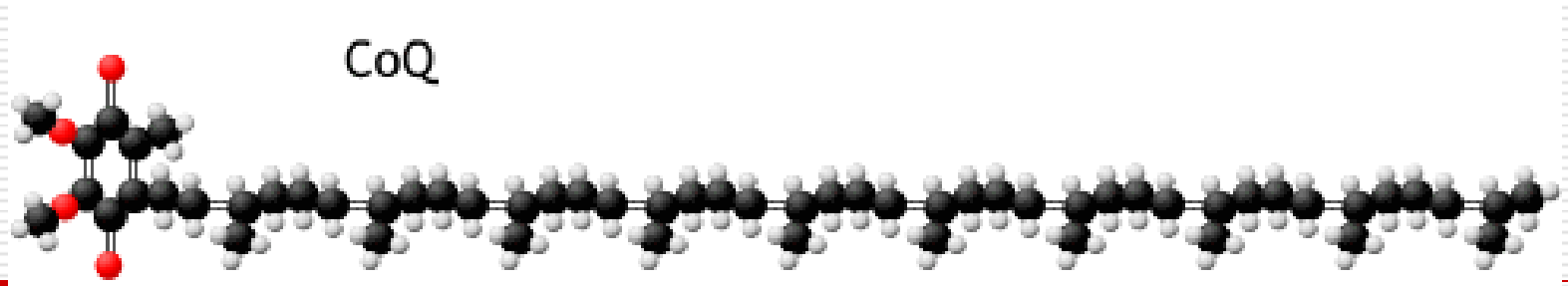
The lipophilic native form of Coenzyme Q0 (left) easily goes to the organic solvent (here dichlorethan DCE, lower phase) when an aqueous solution of Coenzyme Q0 is mixed with DCE, the new synthesized form (right) on the contrary stays in the aqueous phase.



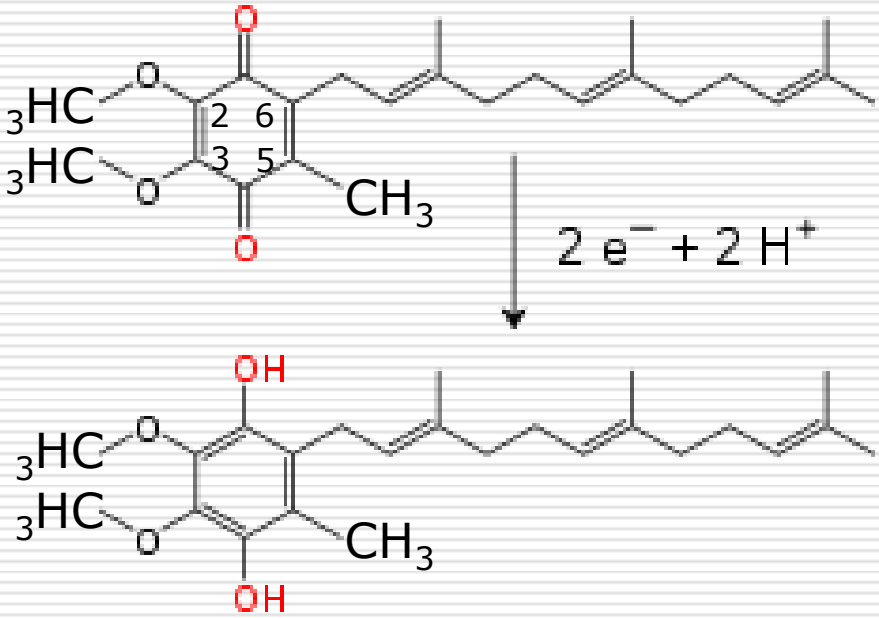




Coenzyme Q10 is a redox mediator in the mitochondrial electron transfer chain (METC) contributing to the mitochondrial ATP production

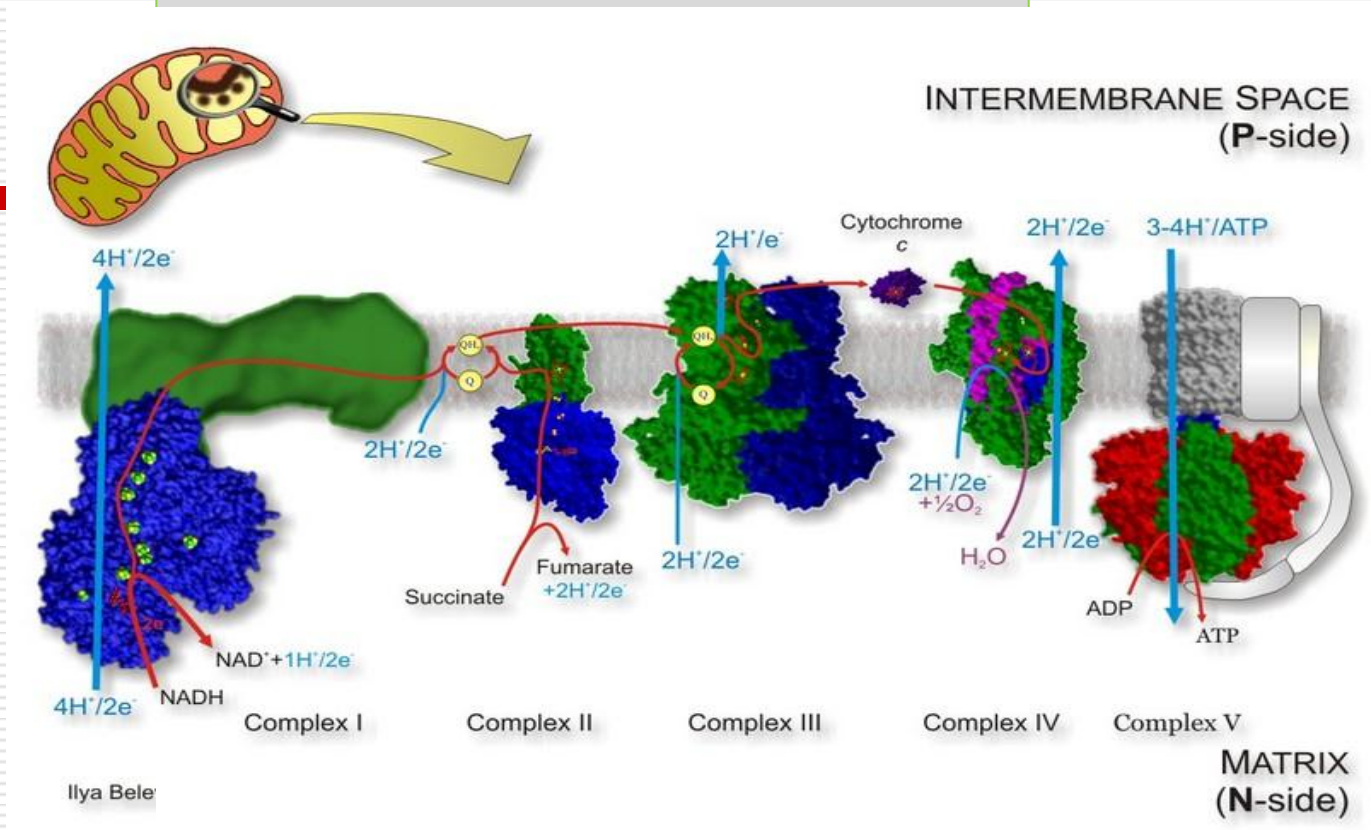


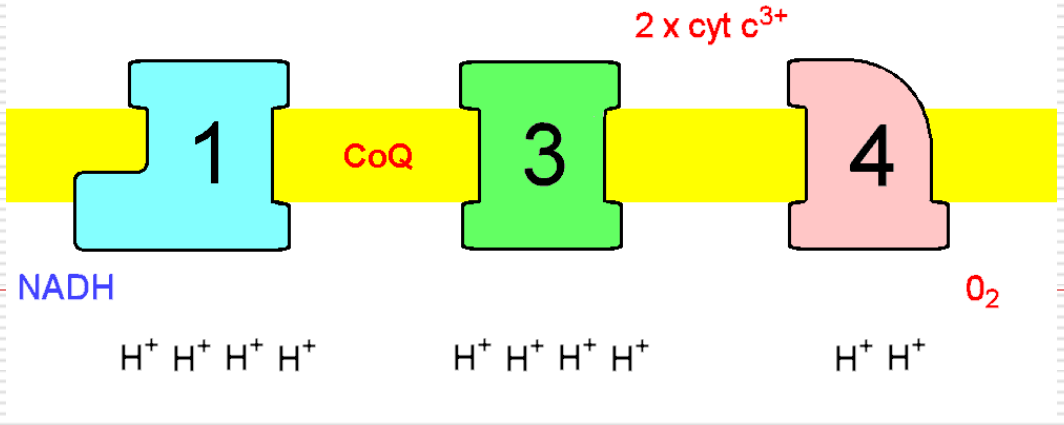
QUINONE (oxidized form)

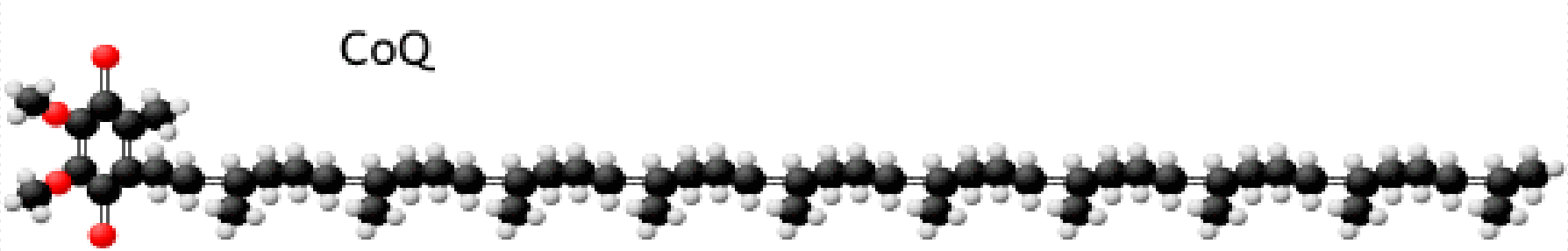


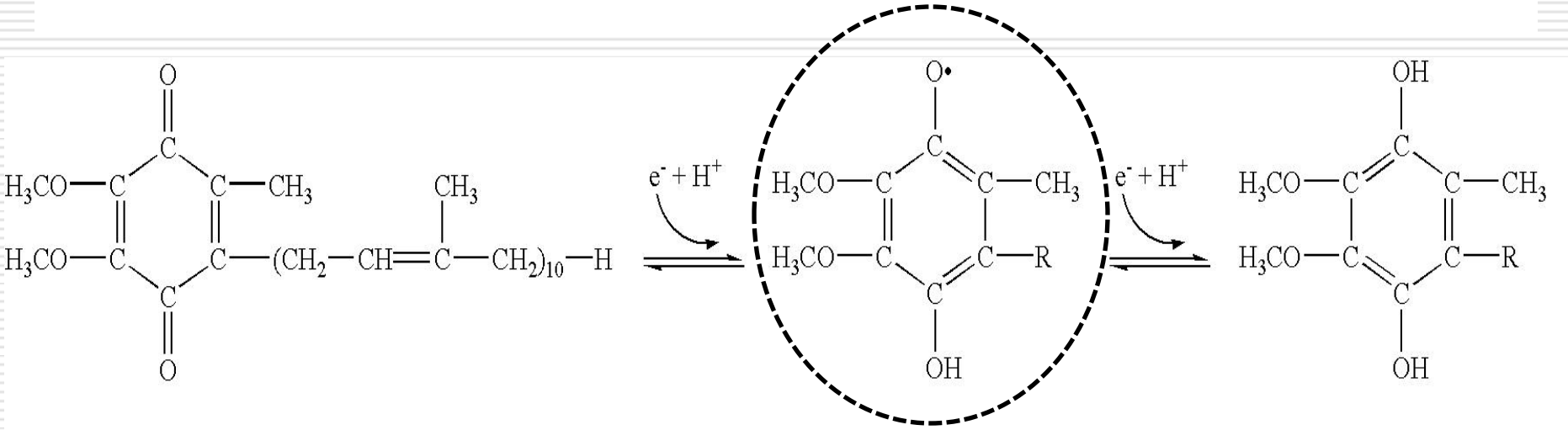
QUINOL (reduced form)

Most of the members of METC are *„**one-electron**” mediators*







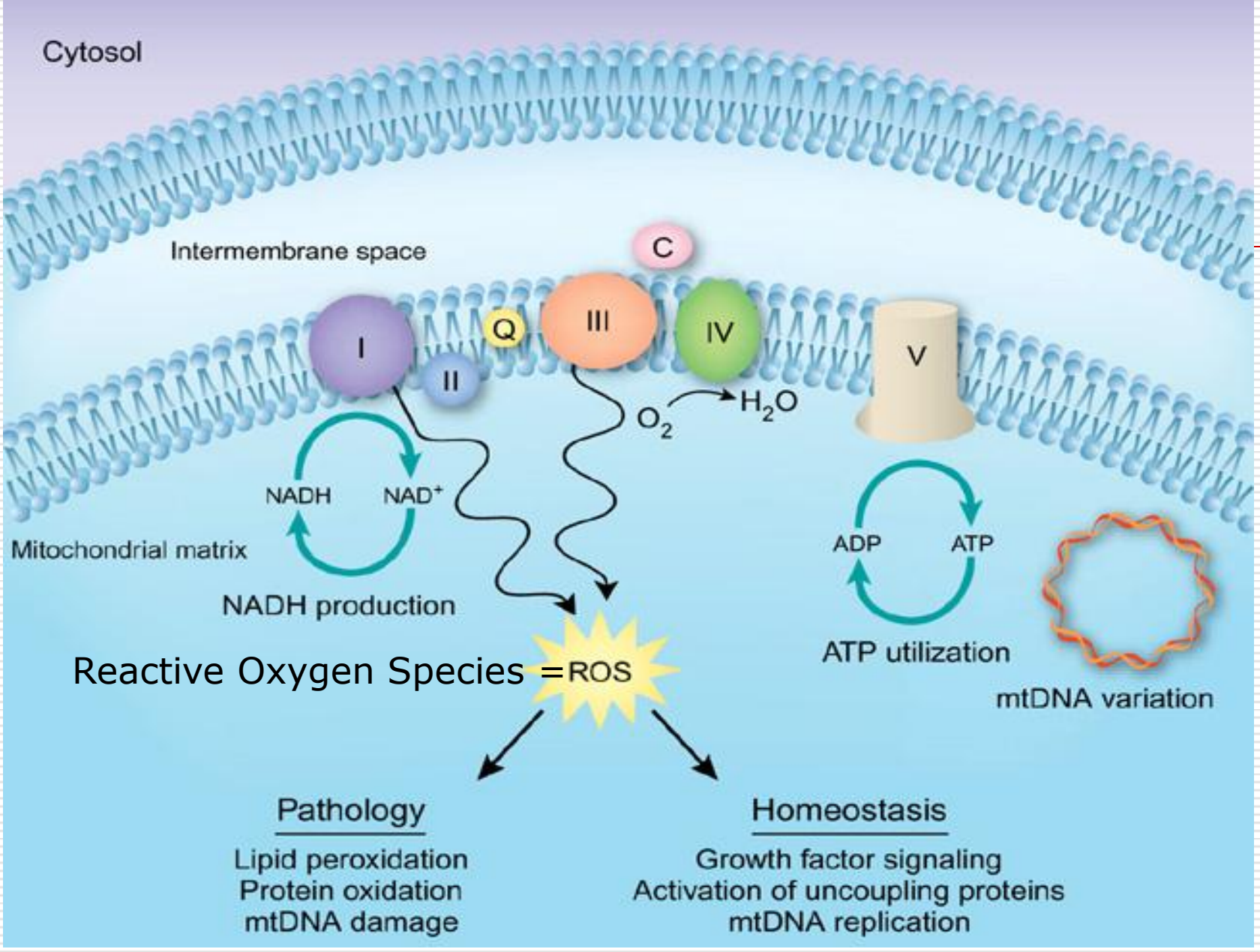


Oxidized form of coenzyme Q
(Q, ubiquinone)

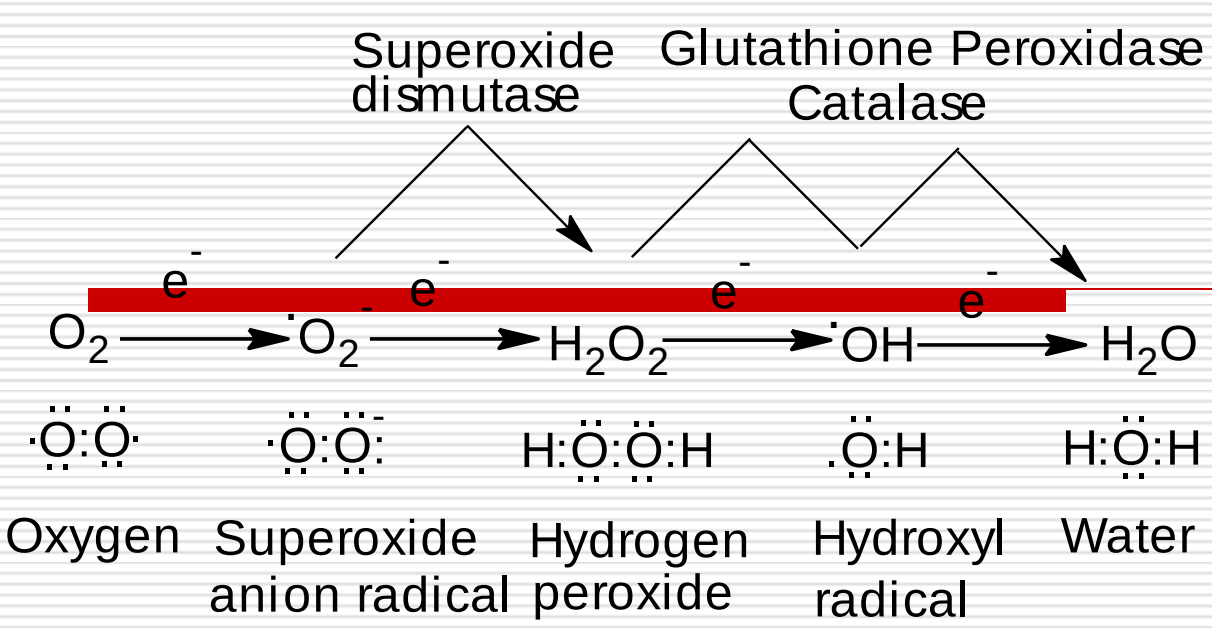
Semiquinone intermediate
(QH•)

Reduced form of coenzyme Q
(QH_2, ubiquinol)

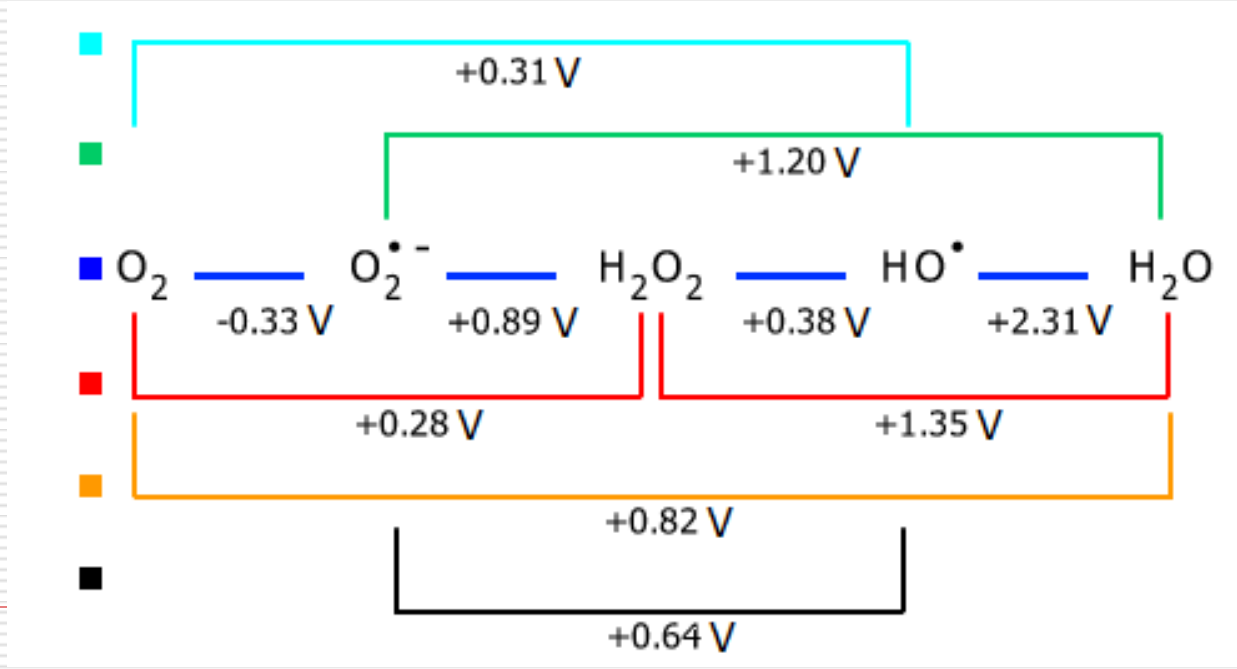
Ubiquinone (Q) is reduced to ubiquinol (QH_2) through a semiquinone intermediate (QH•).

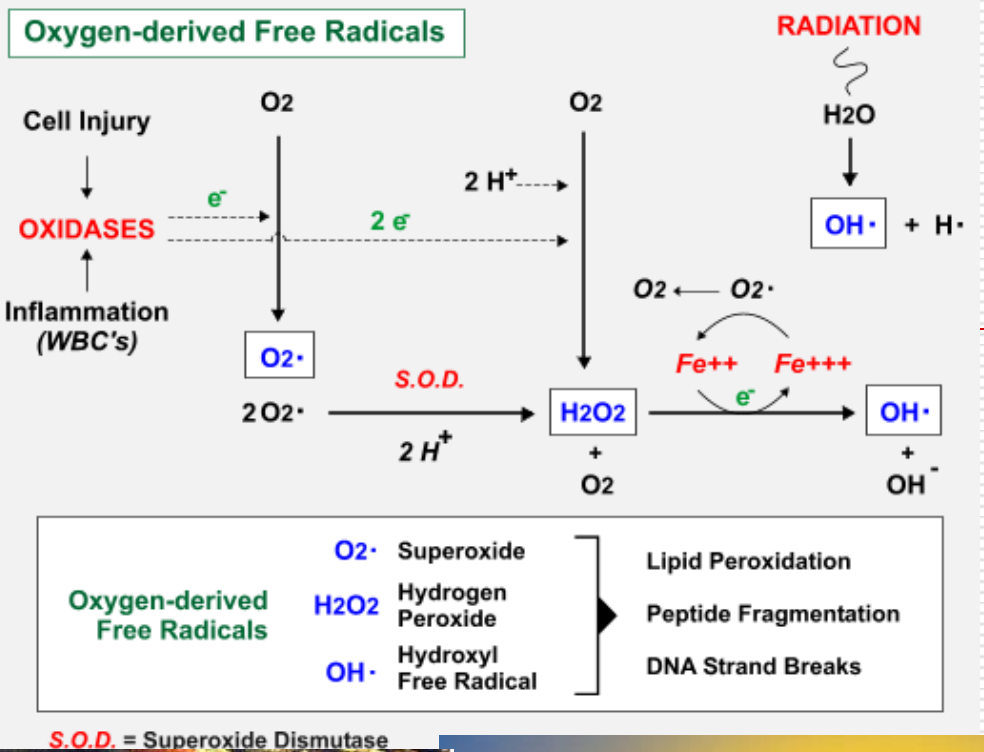


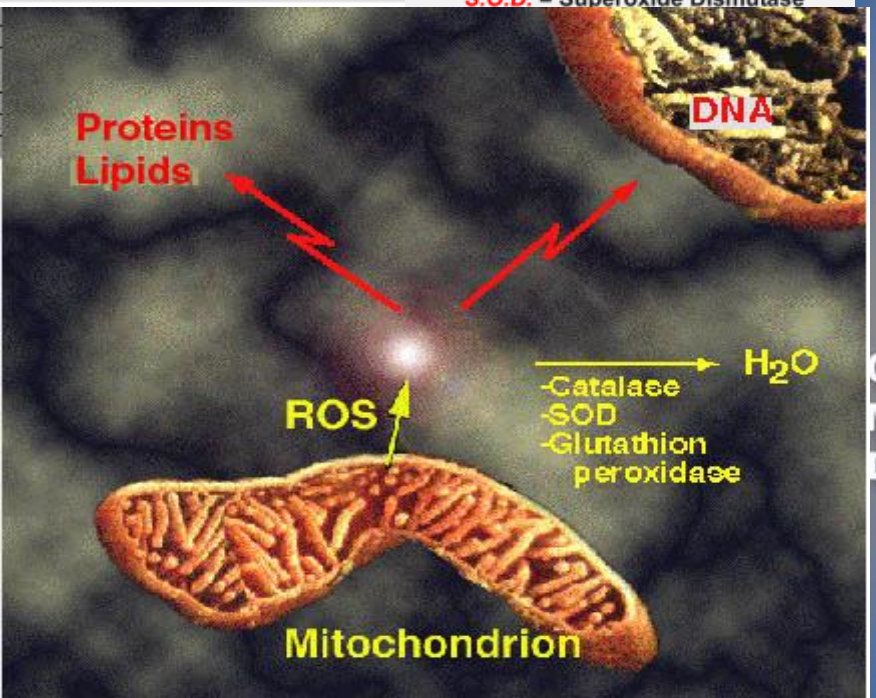
Mitochondrial **electron transport chain** is a major **source** of **highly dangerous ROS**!!!

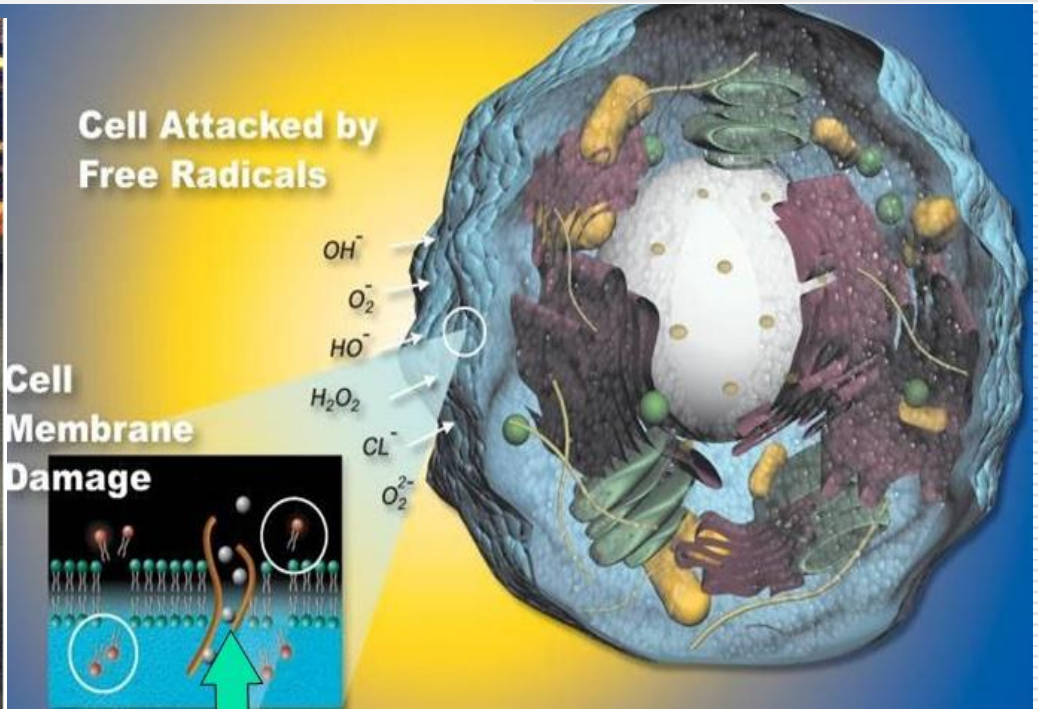


Structures of the most common Reactive Oxygen Species evaluated from O_2

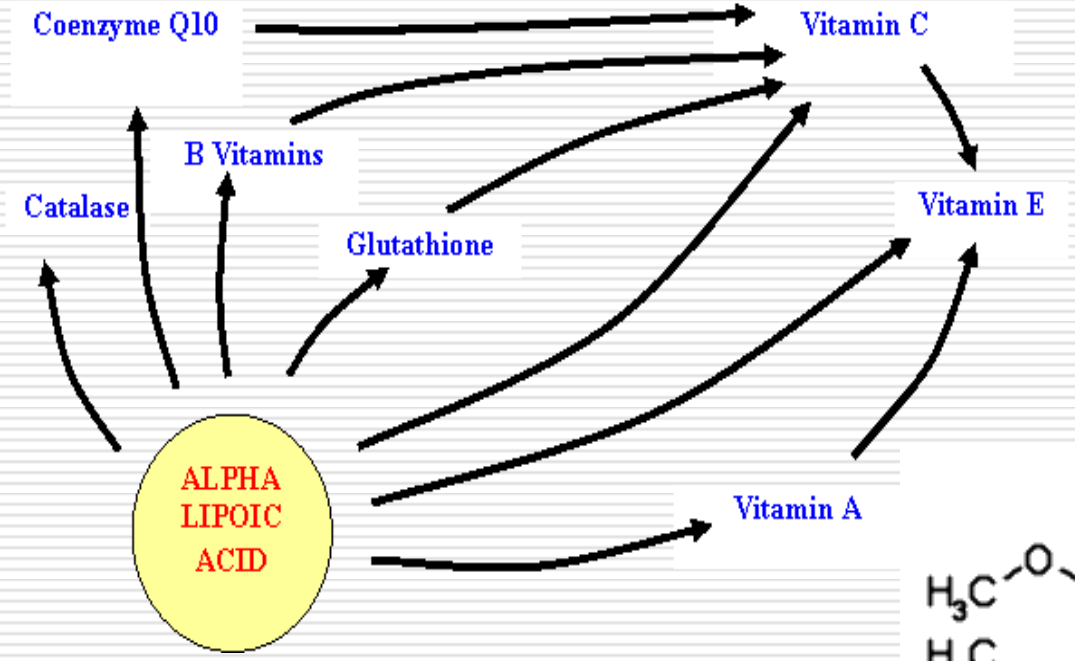


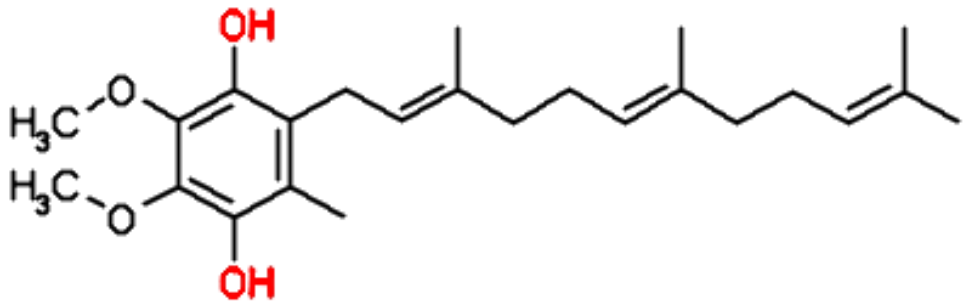

Oxygen-derived Free Radicals
RADIATION
Cell Injury
O2
O2
H2O
e-
2 H+
OXIDASES
2 e-
OH· + H·
Inflammation
(WBC's)
O2 ← O2·
O2·
Fe++ Fe+++
S.O.D.
e-
2 O2·
H2O2
OH·
2 H+
+
O2
+
OH-
Oxygen-derived Free Radicals
O2· Superoxide
H2O2 Hydrogen Peroxide
OH· Hydroxyl Free Radical
Lipid Peroxidation
Peptide Fragmentation
DNA Strand Breaks
S.O.D. = Superoxide Dismutase


Proteins
Lipids
DNA
ROS
H2O
-Catalase
-SOD
-Glutathion peroxidase
Mitochondrion


Cell Attacked by Free Radicals
OH-
O2-
HO-
H2O2
CL-
O2 2-
Cell Membrane Damage

Effective „**cure**“ *against* **ROS** are the **Antioxidants**



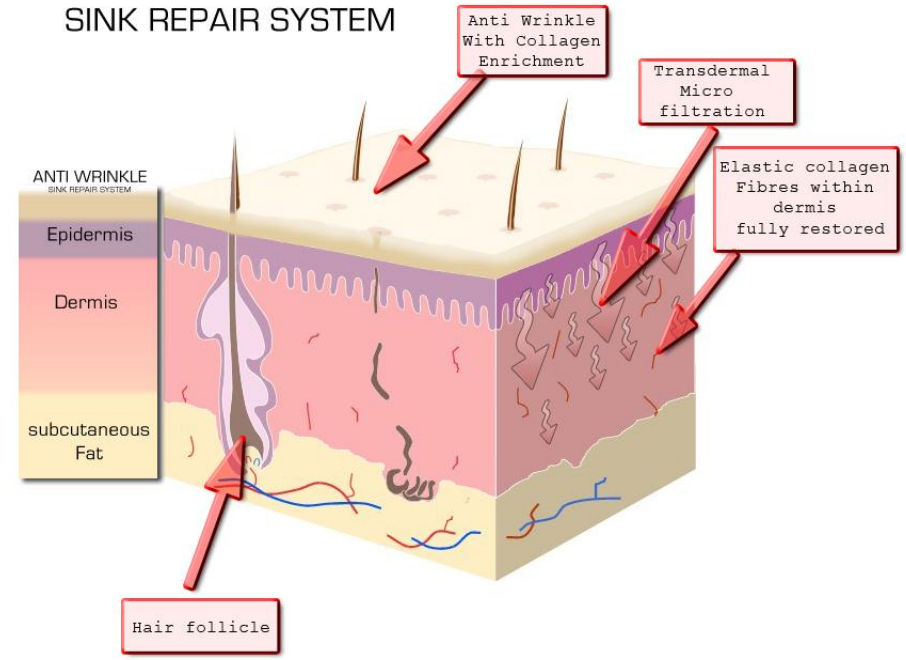


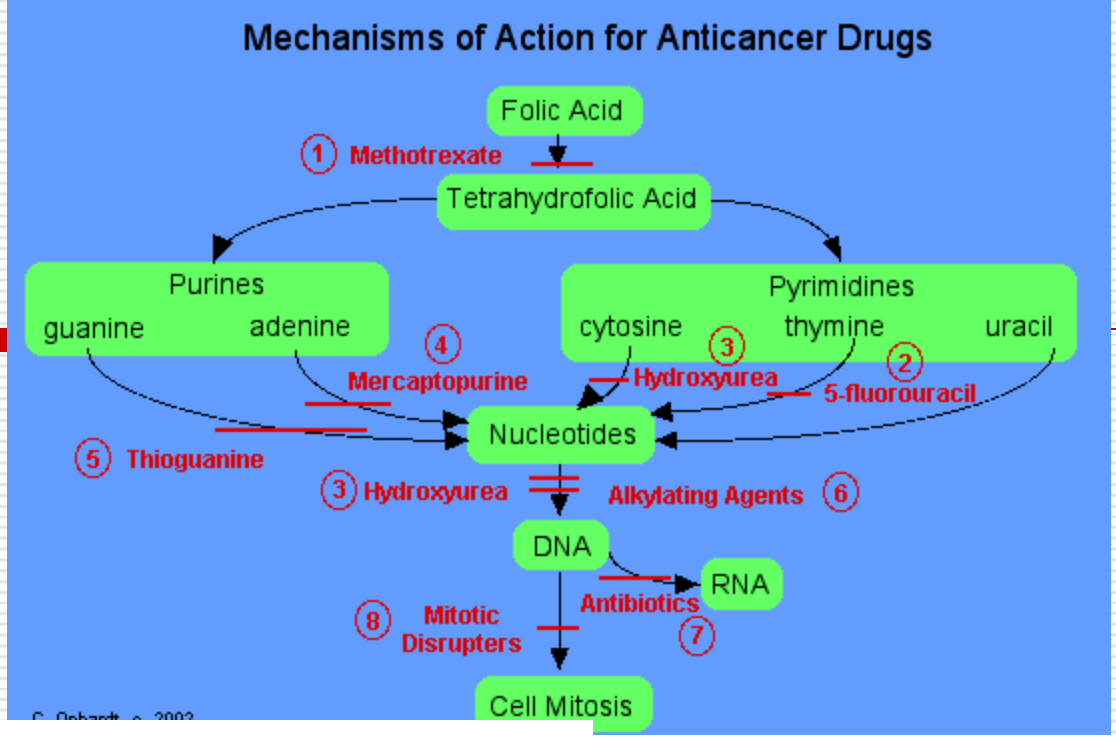
Ubiquinol-moderate antioxidant


Coenzyme Q10


NIVEA
VISAGE
ANTI-WRINKLE Q10plus
LIGHT DAY CREAM


NIVEA
VISAGE
ANTI-WRINKLE Q10plus
NIGHT CREAM


ANTI WRINKLE
SINK REPAIR SYSTEM
Anti Wrinkle With Collagen Enrichment
Transdermal Micro filtration
Elastic collagen Fibres within dermis fully restored
ANTI WRINKLE
SINK REPAIR SYSTEM
Epidermis
Dermis
subcutaneous Fat
Hair follicle



http://www.tumorx.com/peer-reviewed-Q10-protocol.html

ANTICANCER RESEARCH *29*: 33-40 (2009)

Antioxidant Effects of Quercetin and Coenzyme Q10 in Mini Organ Cultures of Human Nasal Mucosa Cells

MAXIMILIAN REITER, KRISTINA RUPP, PHILIPP BAUMEISTER, SABINA ZIEGER and ULRICH HARRÉUS

Department of Otorhinolaryngology / Head and Neck Surgery, Ludwig Maximilians University, Munich, Germany

Abstract. *Background: Oxidative DNA damage is a known risk factor of head and neck cancer. Antioxidants, such as coenzyme Q10 (CoQ_{10}) and quercetin, a member of flavonoids present in red wine and tea, are thought to play a significant role in protecting cells from oxidative stress induced by reactive oxygen species (ROS). The aim of this study was to investigate antioxidant effects of quercetin and CoQ_{10} on mini organ cultures (MOCs) of human nasal mucosa. Materials*

prevention plays an important role in this subject. In this context, antioxidants and may help to prevent DNA damage caused by reactive oxygen species (ROS) (2) and flavonoids represent one of the most important group of substances. These low molecular weight compounds are found in seeds, citrus fruits, olive oil, tea and red wine, and to have possible antioxidant activities *in vivo* (3, 4). Antiproliferative effects on human cancer cell lines have been shown in various

CoQ10 and Cancer

(NCI Report)

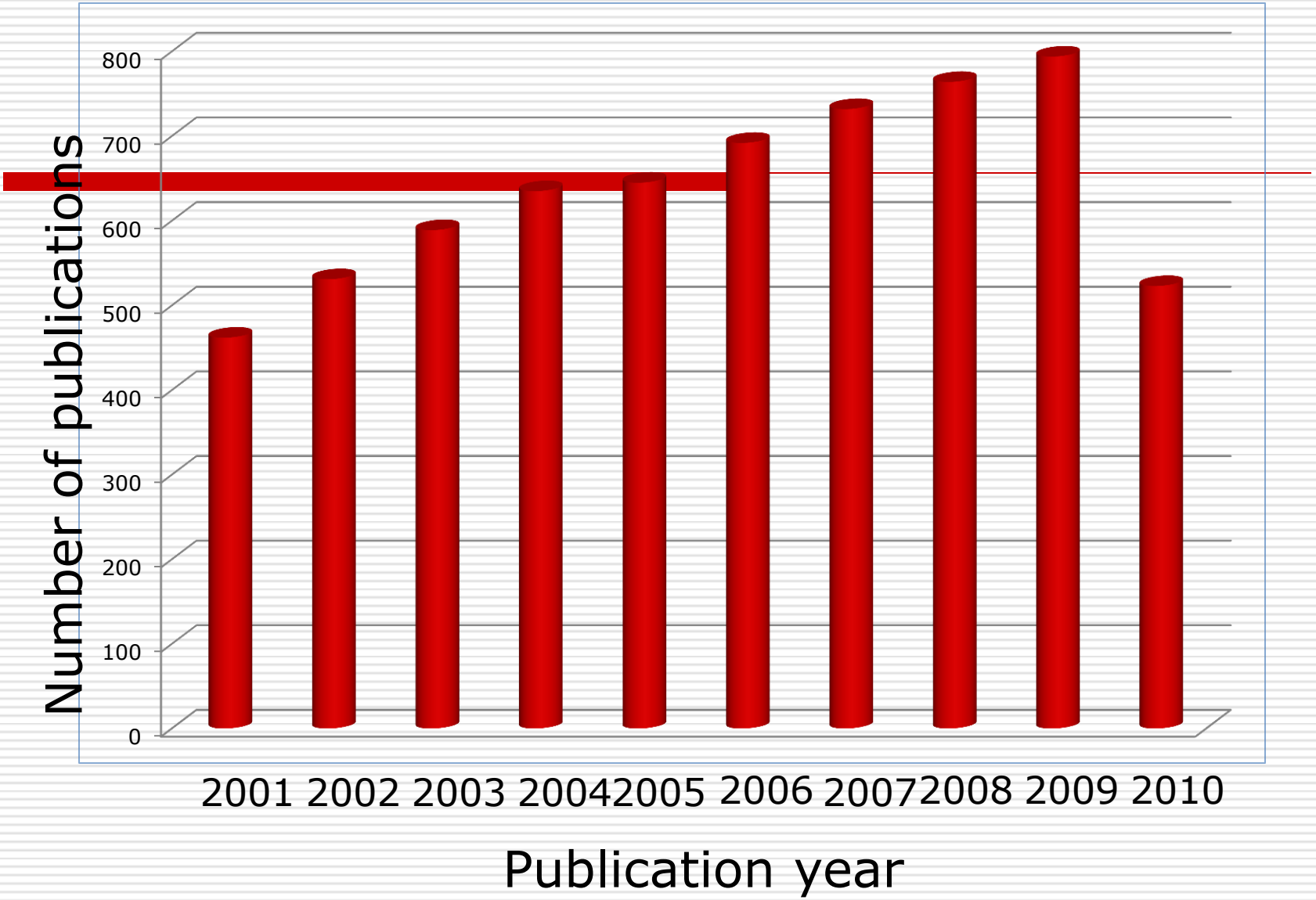
The following information is from a U.S. Government web site (the National Cancer Institute at the National Center for Complementary and Alternative Medicine).

What is the history of the discovery and use of coenzyme Q10 as a complementary or alternative treatment for cancer?

Coenzyme Q10 was first identified in 1957. Its chemical structure was determined in 1958. Interest in coenzyme Q10 as a potential treatment for cancer began in 1961, when a deficiency of the enzyme was noted in the blood of cancer patients. Low blood levels of coenzyme Q10 have been found in patients with myeloma, lymphoma, and cancers of the breast, lung, prostate, pancreas, colon, kidney, and head and neck.

Studies have yielded information about how coenzyme Q10 works in the body to produce energy and act as an antioxidant. Some studies have suggested that coenzyme Q10 stimulates the immune system and increases resistance to disease. In part because of this, researchers have theorized that coenzyme Q10 may be useful as an adjuvant therapy for cancer. (Adjuvant therapy is treatment given following the primary treatment to enhance the effectiveness of the primary

The number of papers on CoQ10 increases permanently



We started working on this project in 2006

J. Phys. Chem. C **2007**, *111*, 6068−6076

Redox Chemistry of Ca-Transporter 2-Palmitoylhydroquinone in an Artificial Thin Organic Film Membrane

Valentin Mirčeski,*,† Rubin Gulaboski,‡ Ivan Bogeski,§ and Markus Hoth§

Institute of Chemistry Faculty of Natural Sciences and Mathematics, Ss Cyril and Methodius University, Skopje, Republic of Macedonia, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre, 687, 4169-007 Porto, Portugal, and Department of Physiology, Saarland University, 66421 Homburg, Germany

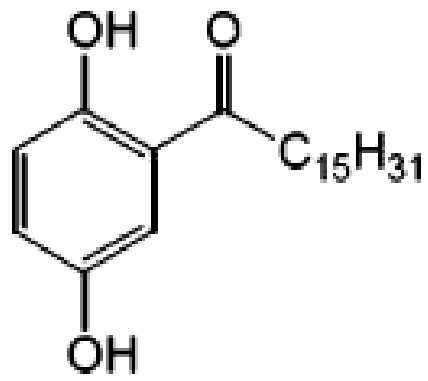
Received: December 14, 2006; In Final Form: January 26, 2007

The redox chemistry of 2-palmitoylhydroquinone (H_2Q), a recently introduced synthetic transmembrane Ca^{2+} transporter, was studied with cyclic and square-wave voltammetry in an artificial thin organic-film membrane sandwiched between a pyrolytic graphite electrode and an aqueous solution. The membrane has a micrometer dimension and consists of the water immiscible organic solvent nitrobenzene, which contains suitable electrolyte and H_2Q as a redox active compound. The potential drop at the electrode/membrane interface is controlled by the potentiostat, whereas the potential drop at the membrane/water interface is dependent on the ClO_4^- concentration, which is present in a large excess in both liquid phases. The redox transformation of H_2Q at the electrode/membrane interface is accompanied by a corresponding ion-transfer reaction at the other side of the membrane. Proton transfer at the membrane/water interface is critical for the redox transformation of H_2Q in the interior of the membrane, as a strong dependence of the voltammetric response on the pH of the aqueous medium was observed. H_2Q undergoes two oxidation processes due to existence of two distinctive redox forms of H_2Q. The electrochemical mechanism can be explained with two tautomer forms of H_2Q formed by migration of a proton between the 1-hydroxyl group and the adjacent carbonyl group of the palmitoyl residue. Both tautomers undergo $2e/2H^+$ distinctive redox transformations to form the quinone form of the studied compound. In the presence of Ca^{2+} in the aqueous phase, voltammetric experiments confirmed the capability of both tautomers to form 1:1 complexes with Ca^{2+} and to extract it into the organic membrane. Upon the oxidation of the complexes, Ca^{2+} is expelled back to the aqueous phase. The studied compound exhibits very similar complexing affinity toward Mg^{2+}, implying that it is not highly selective for transmembrane Ca^{2+} transport.

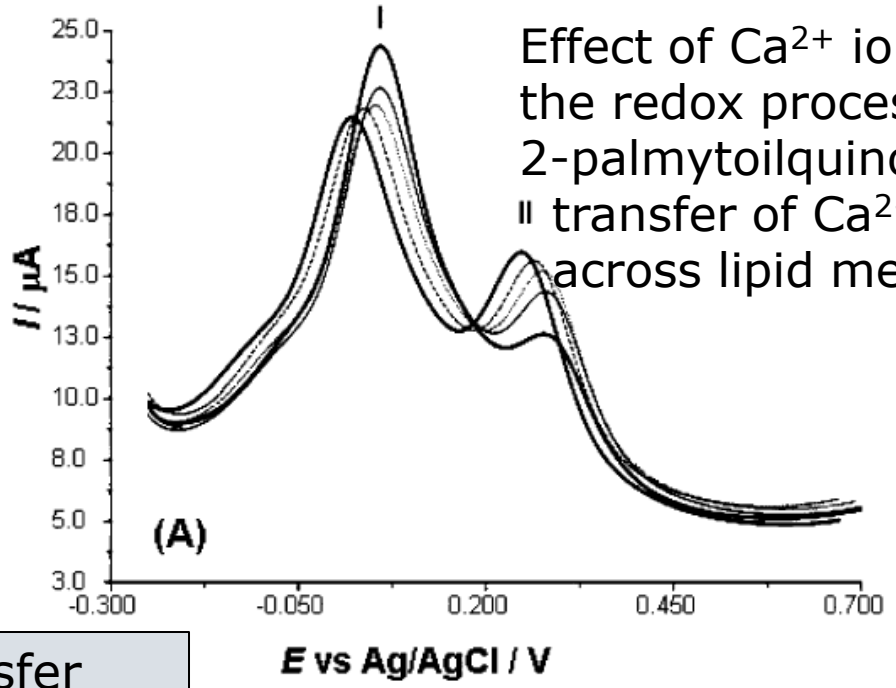
1. Introduction

Organic compounds containing a benzoquinone/hydroquinone moiety are subjects of longstanding research efforts in various scientific areas due to their high relevance to biochemical systems. The most important example is ubiquinone-10 (also called coenzyme Q), which plays a critical role in the respiratory chain of mitochondria.[1] Embedded in the inner mitochondria membrane, ubiquinone-10 serves as an electron shuttle and a proton pump, generating a proton and potential gradient at the inner mitochondrion membrane. The energy conserved in a form of a potential gradient is further used for synthesis of adenosine-triphosphate. The redox chemistry of the quinone/hydroquinone

Electrochemical techniques are very well suited for characterizing quinone-like compounds.[2−9] As most of the physiologically active quinones are lipophilic, electrochemical methods in nonaqueous medium have been developed.[4,6,8] Particularly important are biomimetic studies in which lipophilic quinone is embedded in a lipid membrane supported on the electrode surface.[3] Liposomes are also suitable for membrane immobilization of lipophilic quinones. In this context, Bennett et al.[10] have recently incorporated the synthetic 2-palmitoylhydroquinone (H_2Q) in a liposome membrane to build an artificial light-driven transmembrane calcium pump. Although the redox chemistry of H_2Q is hardly known, these authors have utilized its redox

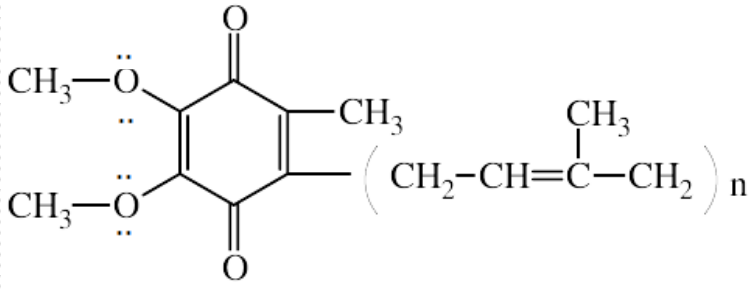


Structure of
2-palmytoilhydroquinone

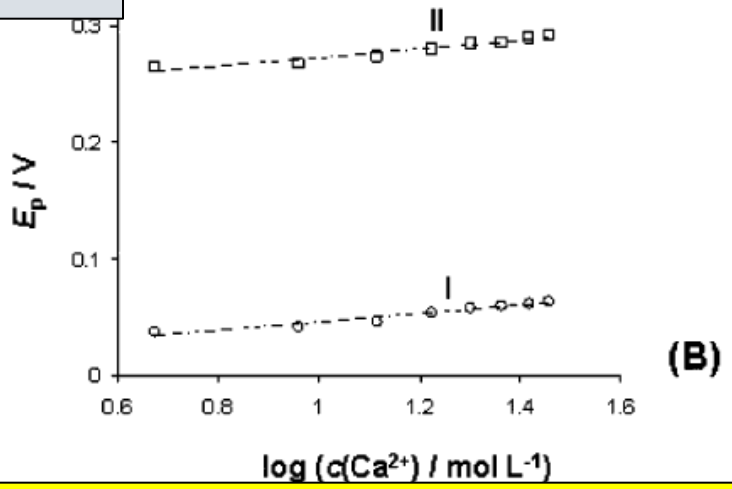


Effect of Ca^{2+} ions to the redox process of 2-palmytoilquinone-transfer of Ca^{2+} across lipid membrane

This compound can bind and transfer Ca^{2+} ions across biomimetic membranes

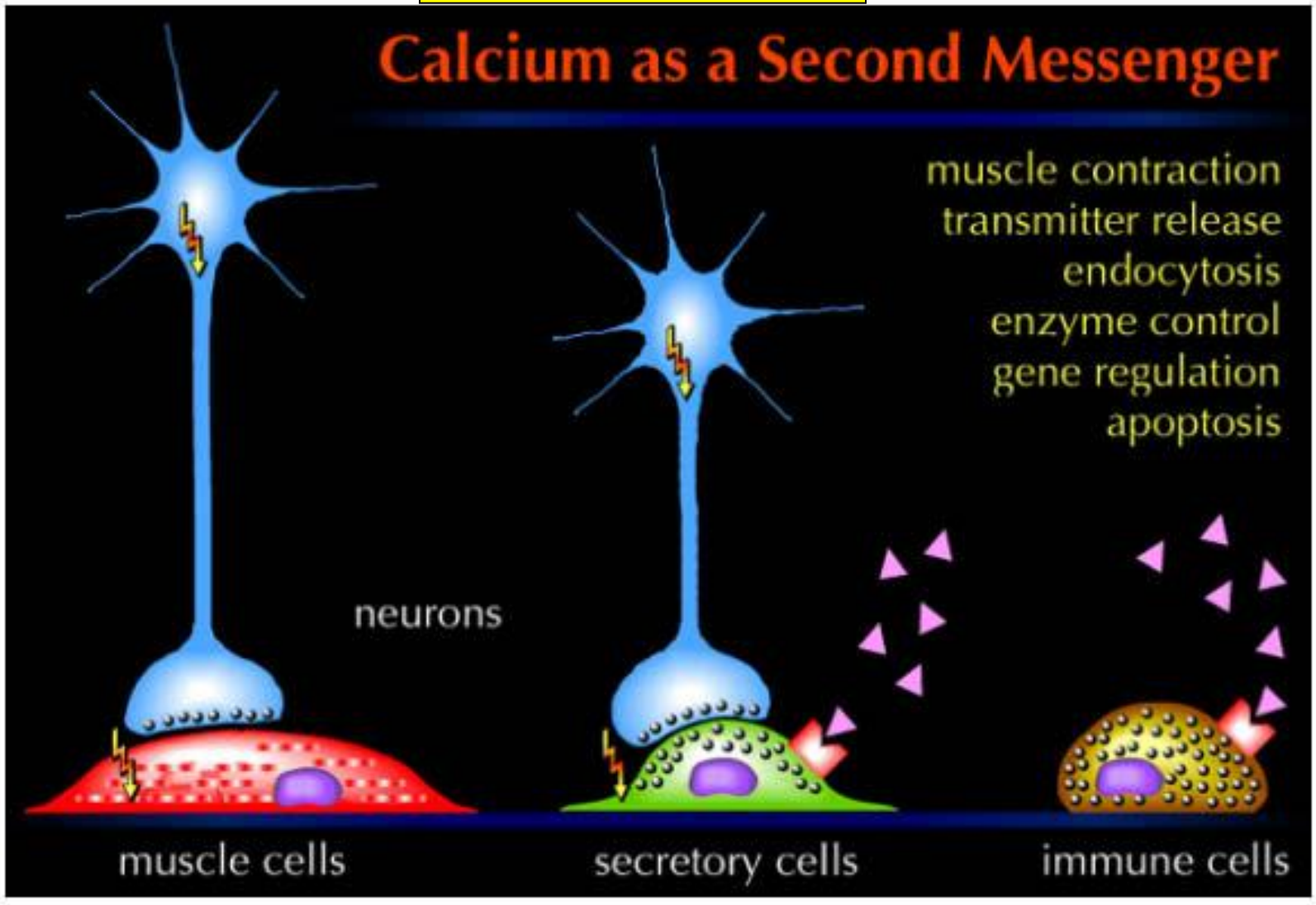


UBIQUINONE (oxidized)



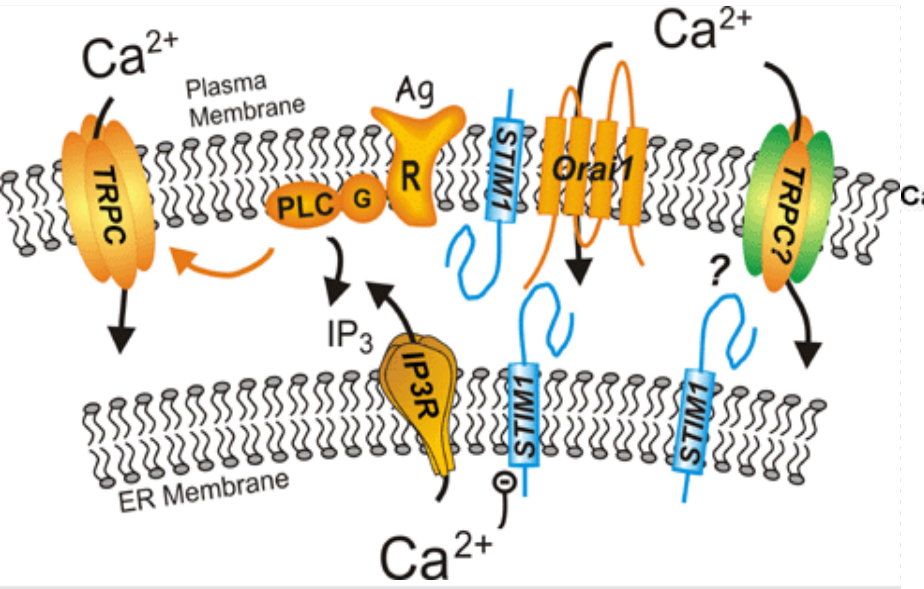
<u>Our Aim</u>: to see whether CoQ's have something to do with Ca^{2+} transfer across mitochondrial membranes

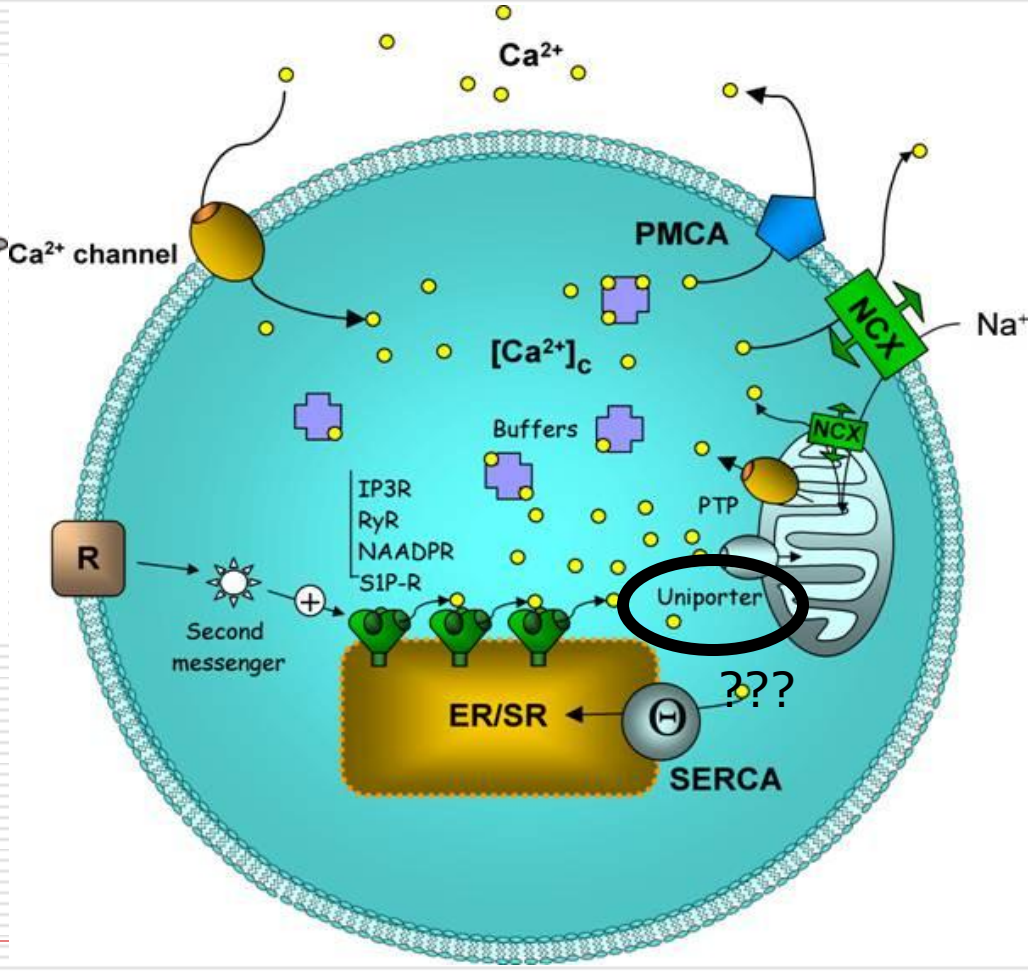
Why Ca^{2+}?

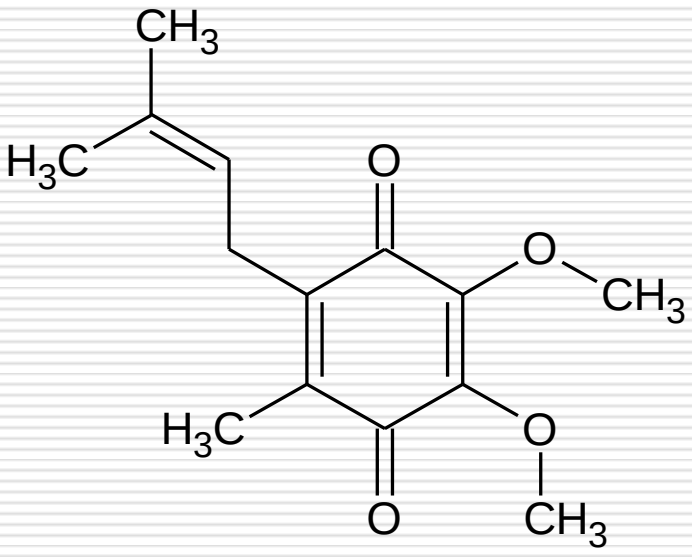


Ca^{2+} -is one of the most important second messengers

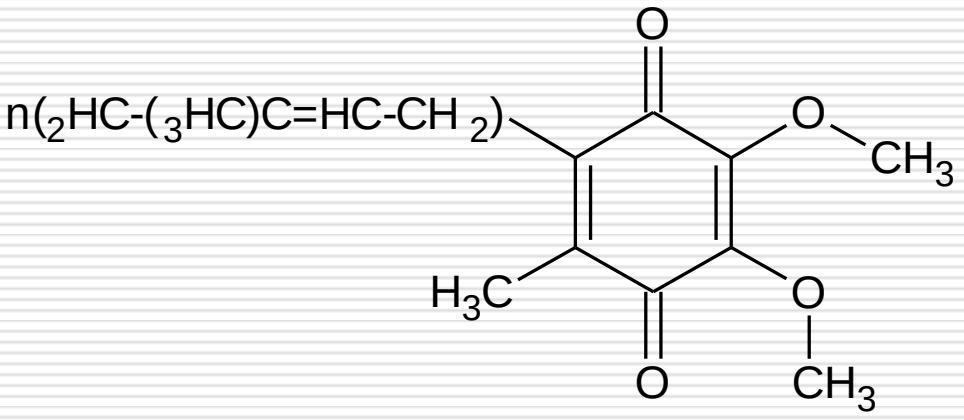
Mechanisms of Ca^{2+} transfer in mitochondria





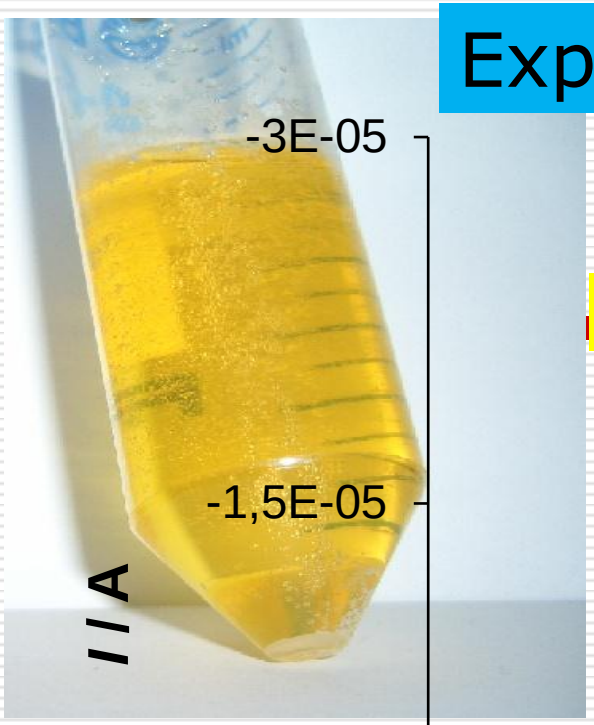


Coenzyme Q_1

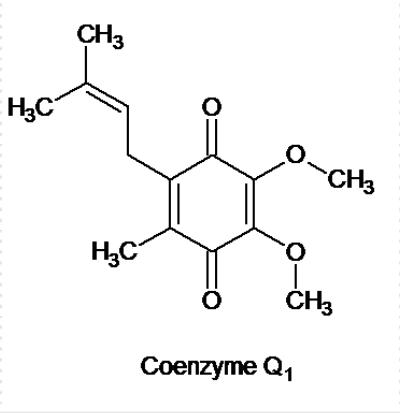


Coenzyme Q_{10}

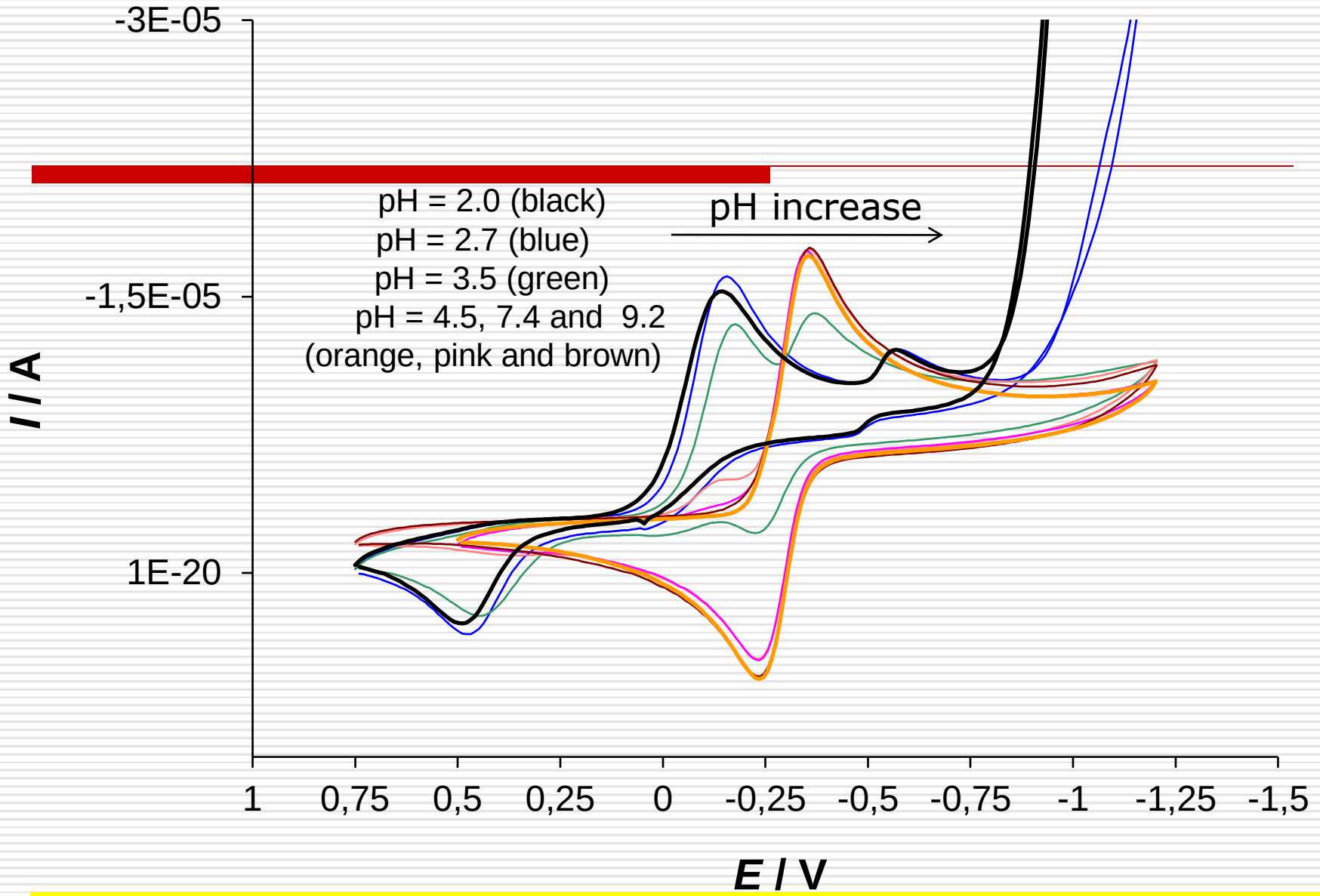
Experiments with Coenzyme Q1-CoQ1



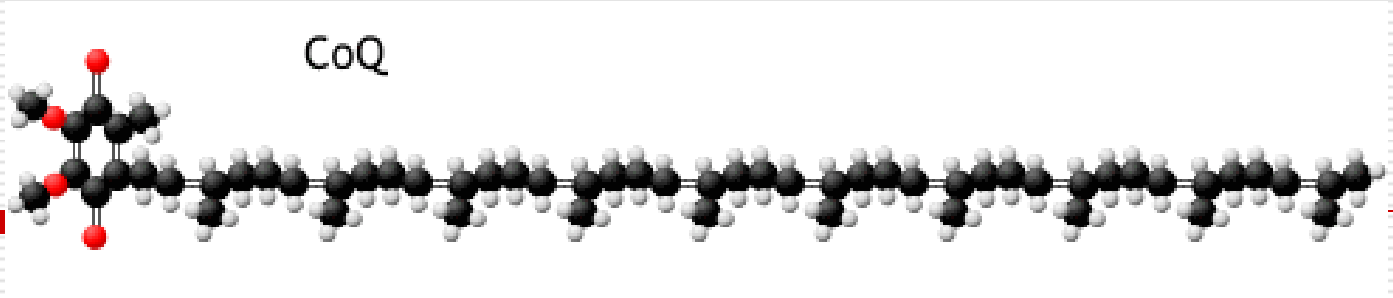
Cyclic voltammogram of 100 μM CoQ1 in pH of 7.00

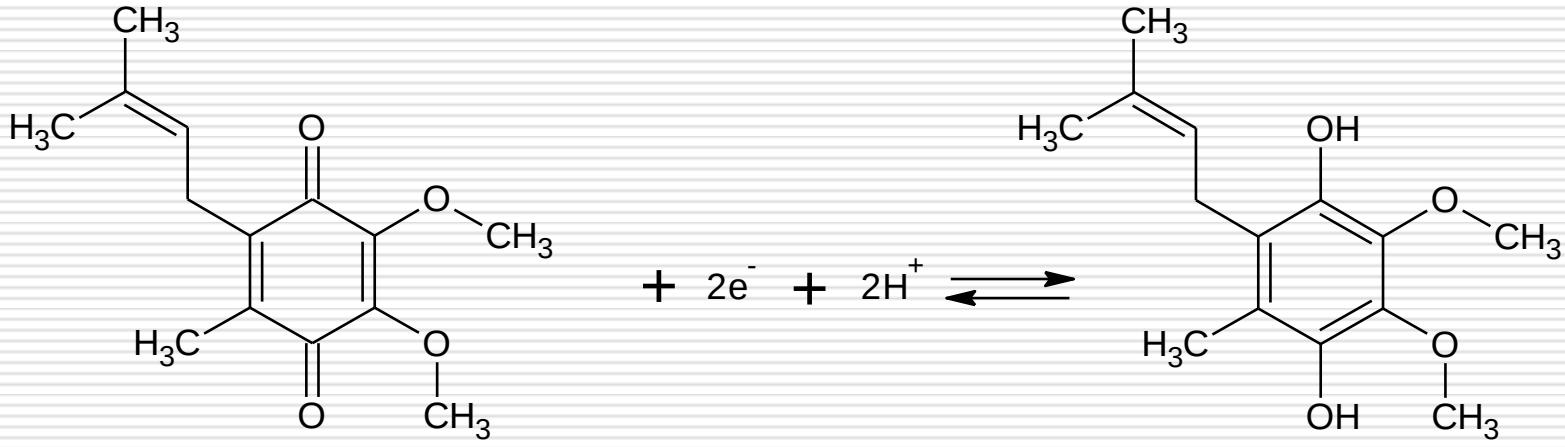


Coenzyme Q_1



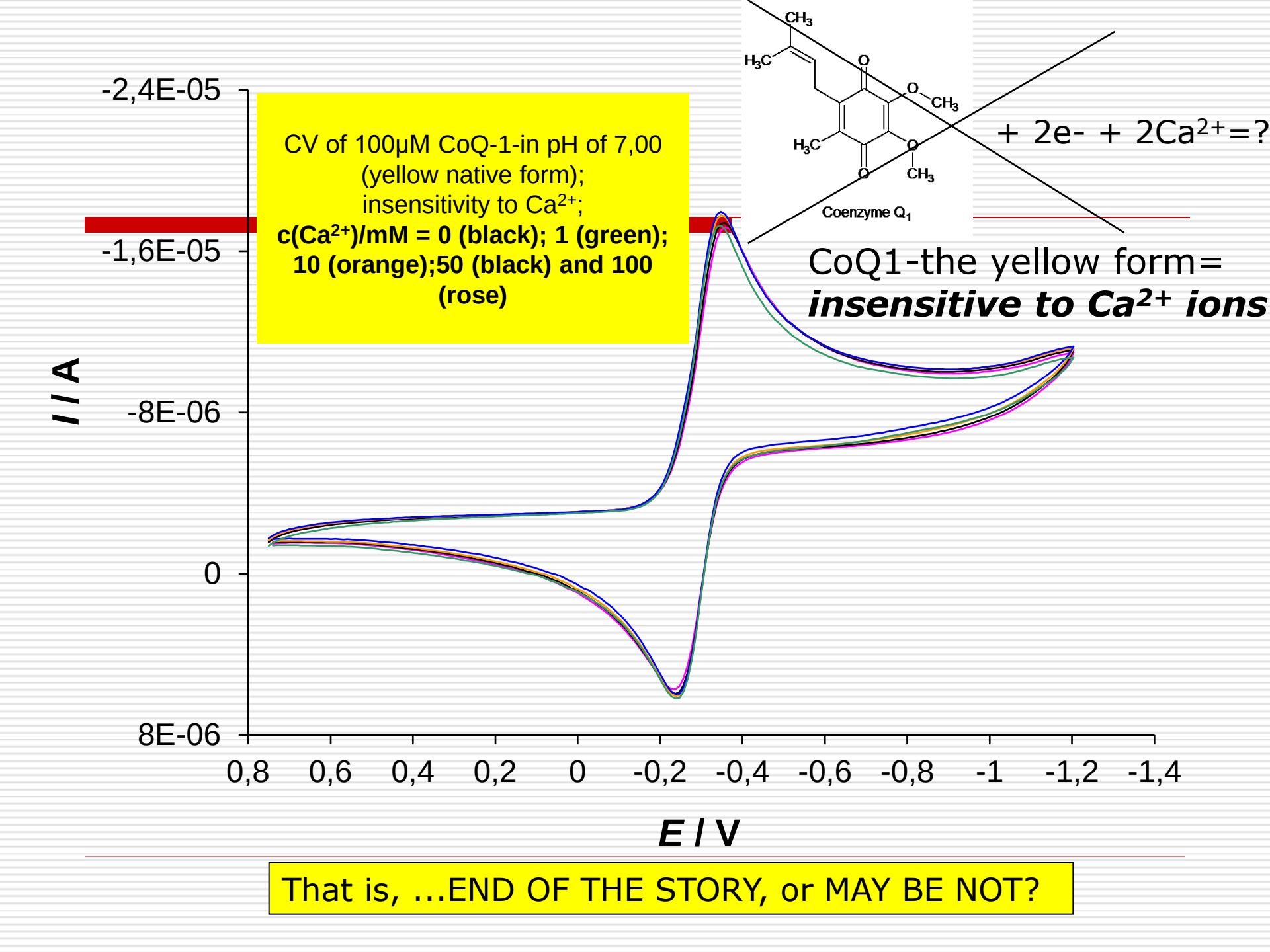
pH dependence of the redox process of CoQ1-the “yellow form” in the pH range from 1 to 9



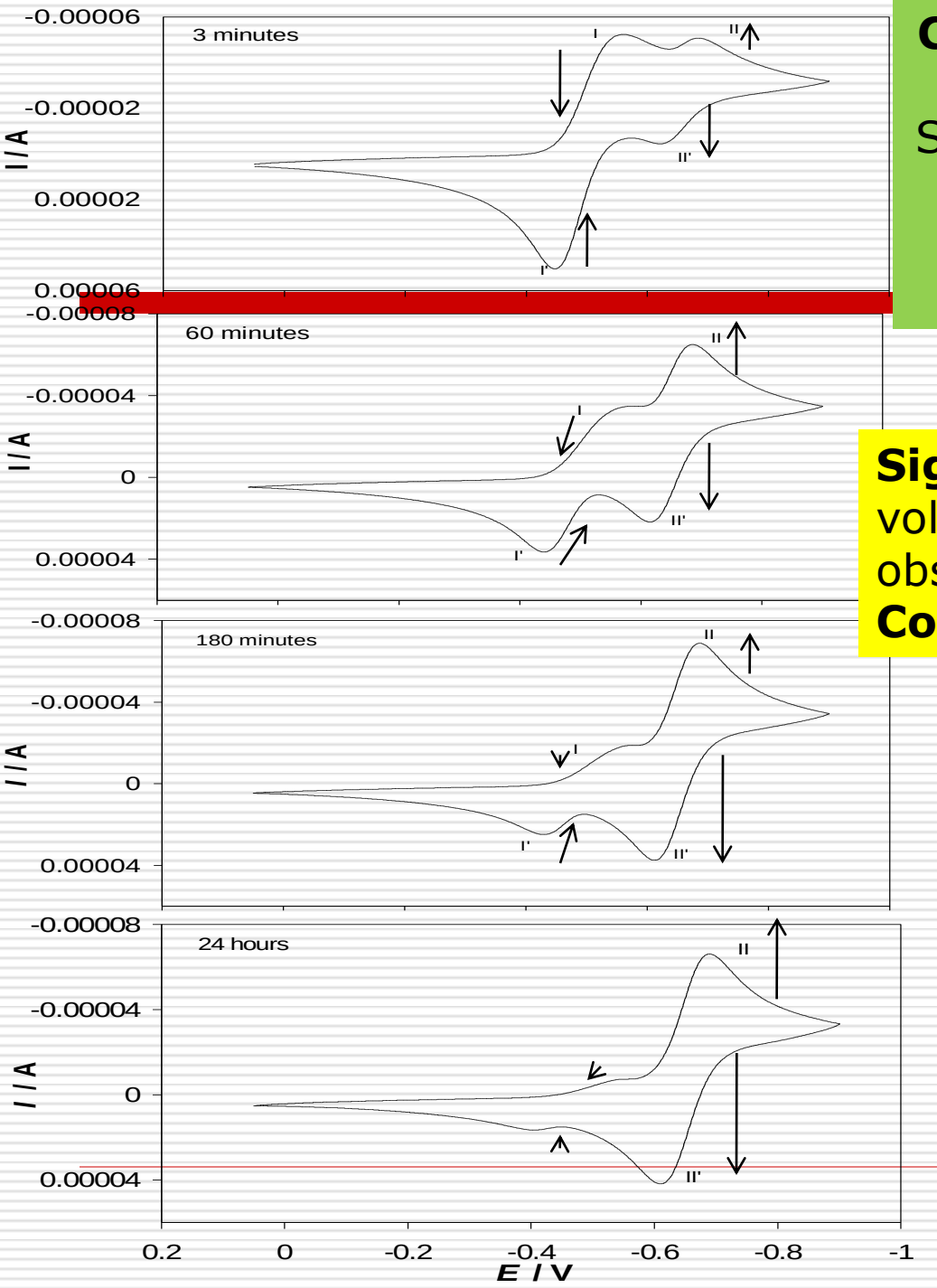


Coenzyme Q_1 (oxidized)

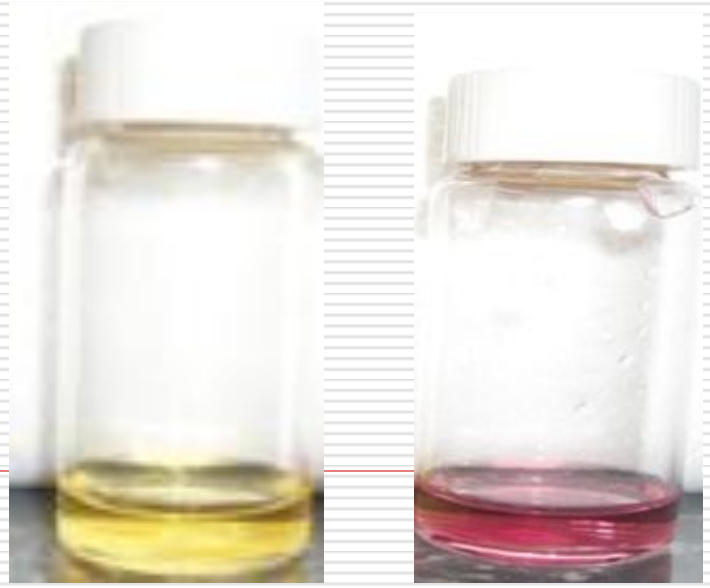
Coenzyme Q_1 (reduced)

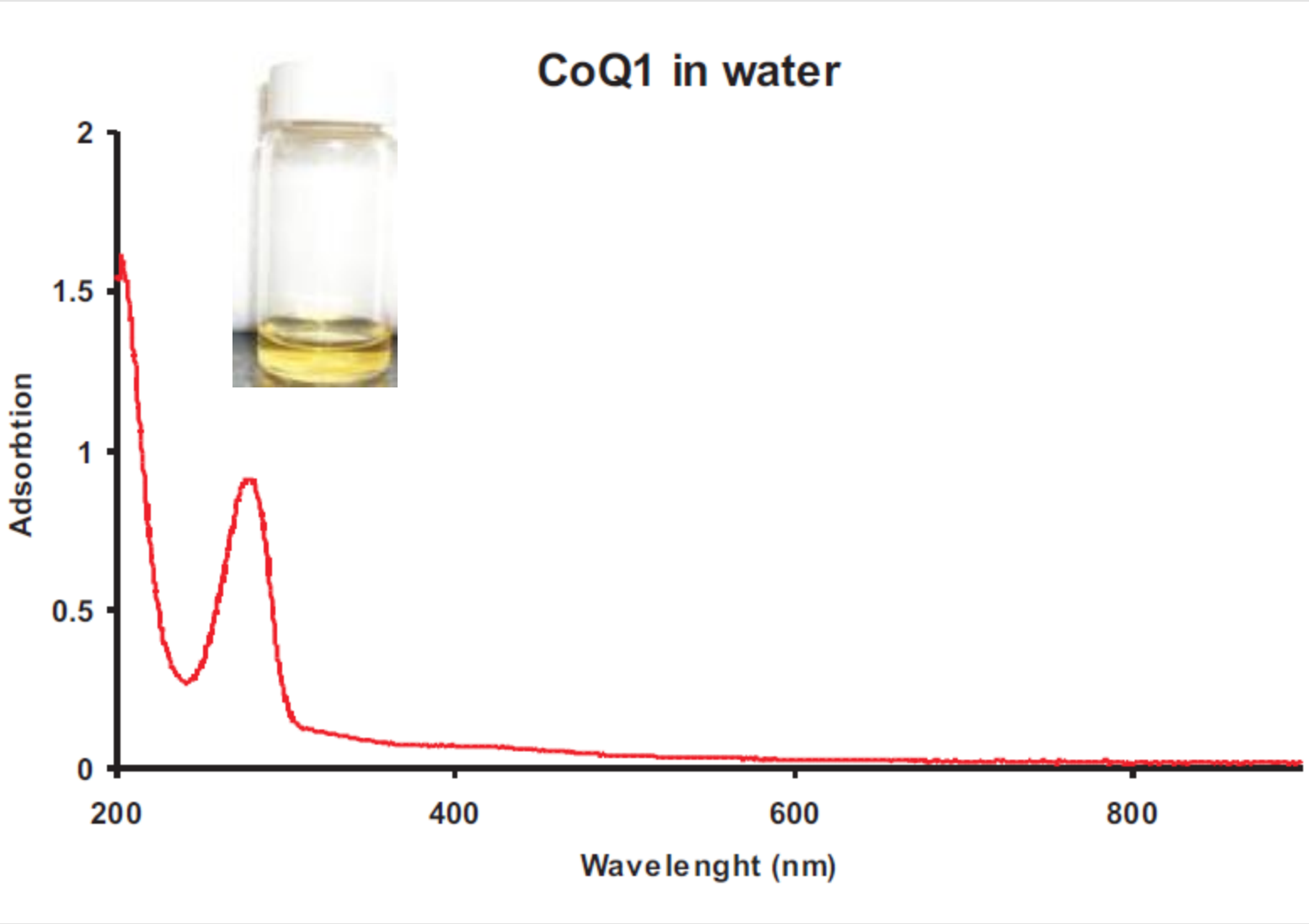

-2,4E-05
-1,6E-05
-8E-06
0
8E-06
I / A
0,8
0,6
0,4
0,2
0
-0,2
-0,4
-0,6
-0,8
-1
-1,2
-1,4
E / V
CV of 100μM CoQ-1-in pH of 7,00 (yellow native form); insensitivity to Ca2+; c(Ca2+)/mM = 0 (black); 1 (green); 10 (orange);50 (black) and 100 (rose)
Coenzyme Q1
+ 2e- + 2Ca2+=?
CoQ1-the yellow form= insensitive to Ca2+ ions
That is, ...END OF THE STORY, or MAY BE NOT?

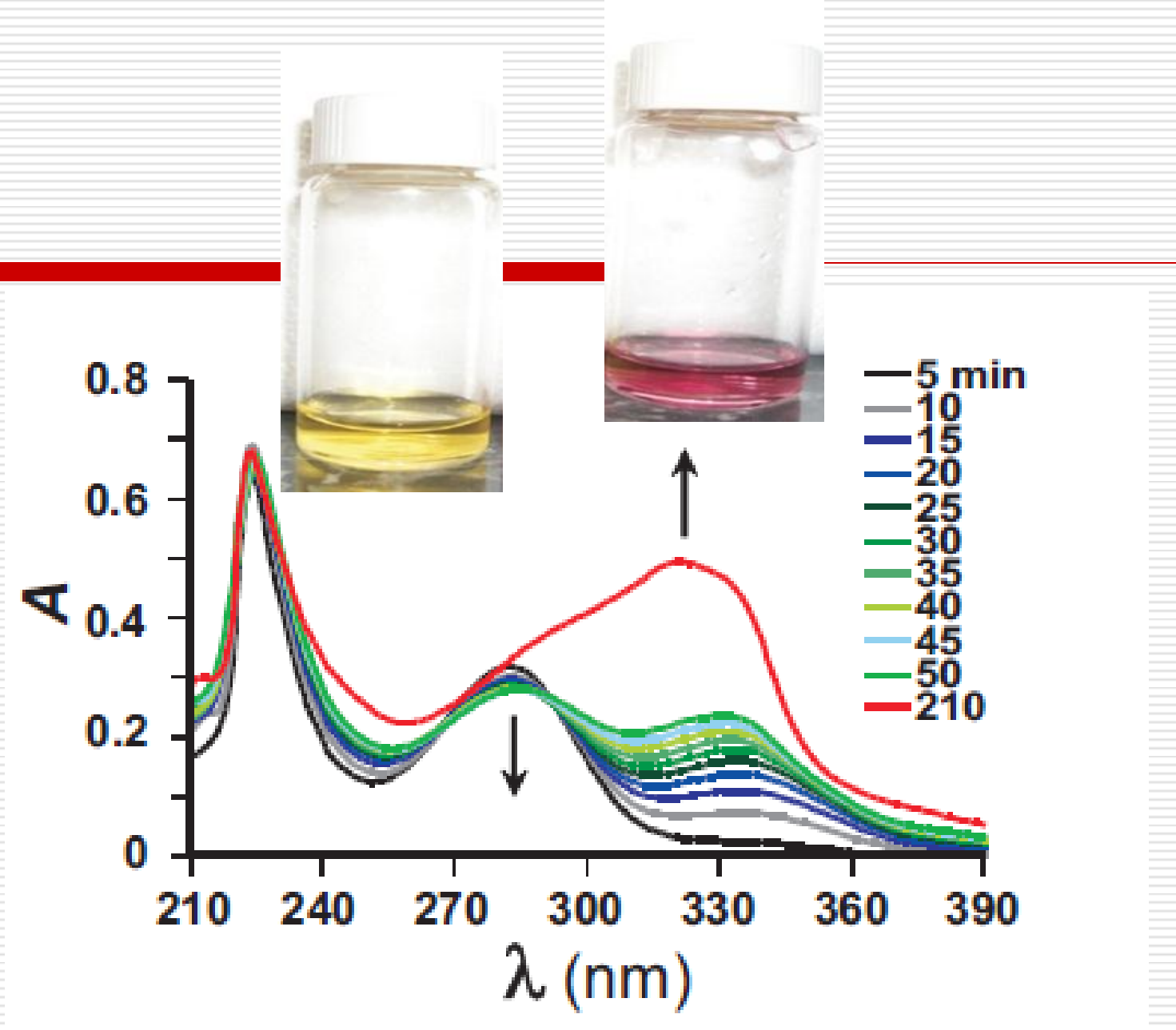
Cyclic voltammograms of CoQ1 recorded in 1 mol/L NaOH.
Scans recorded after 3 min (1) 1 h (2) 3 h (3) and 24 h (4).
Scan rate of 30 mV/s,
c(CoQ1) = 0.075 mmol/L.



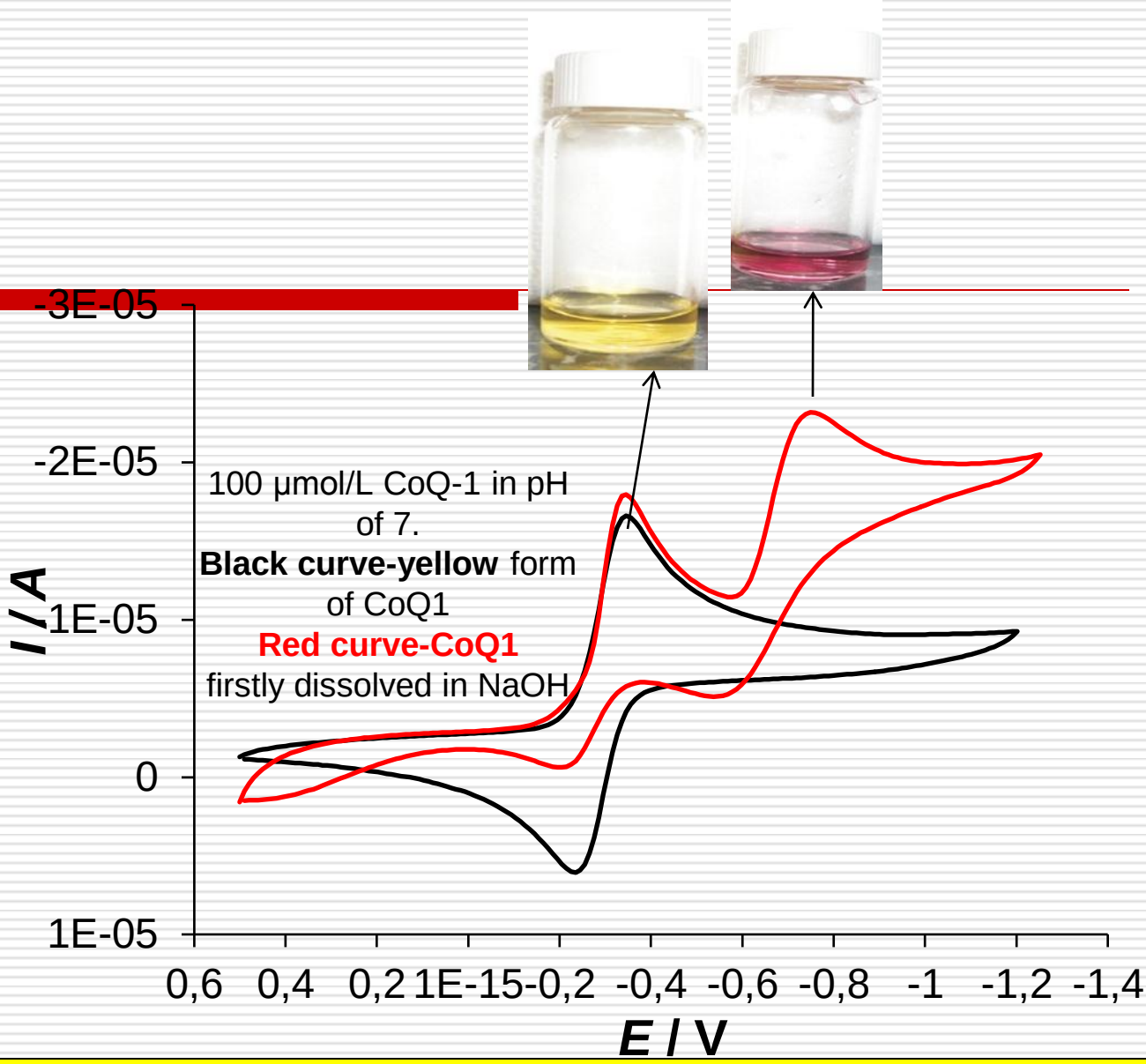
Significant changes in the voltammetric responses have been observed **when CoQ1 was dissolved in 1M NaOH**!



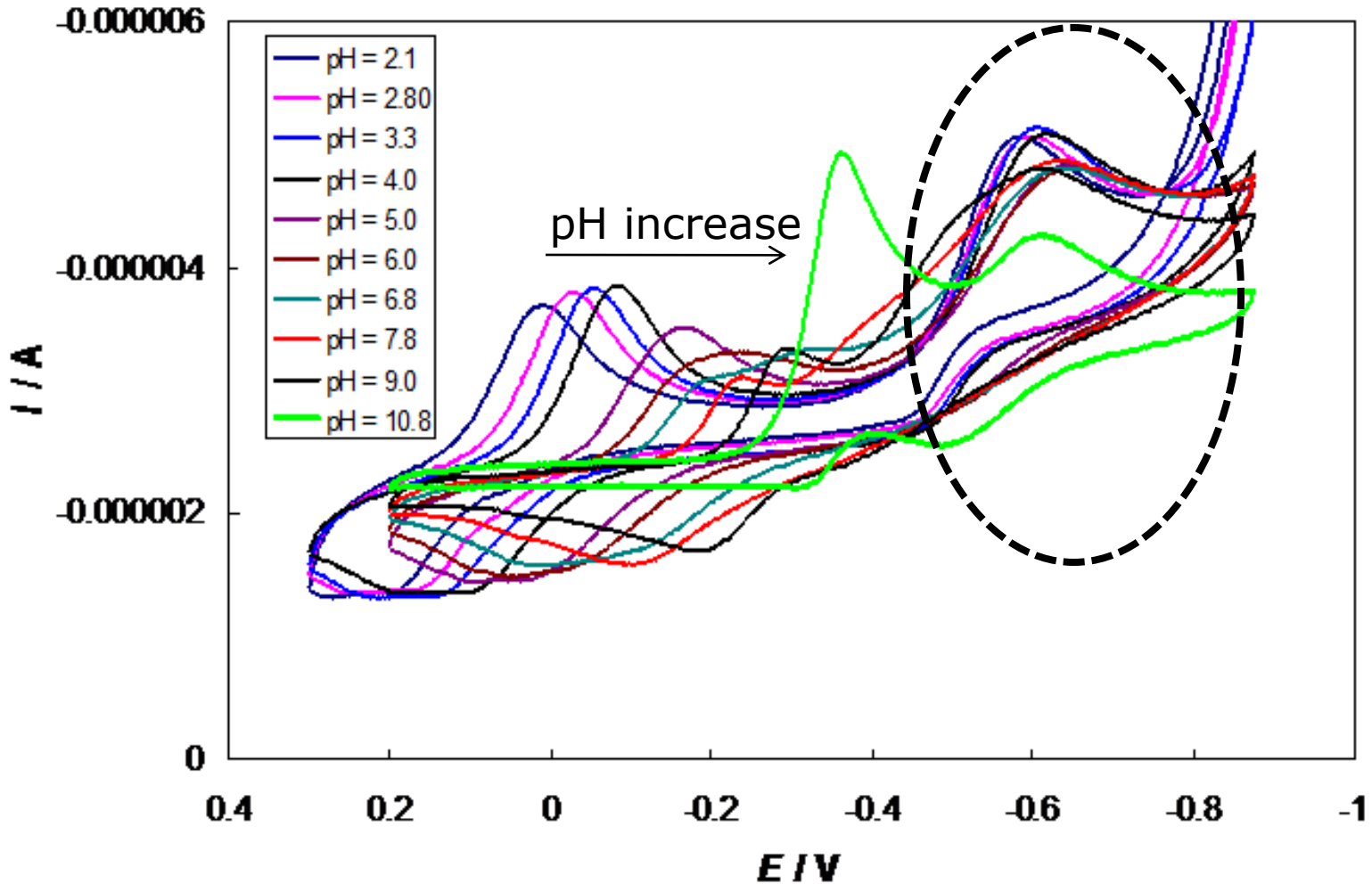

CoQ1 in water
2
1.5
1
0.5
0
Adsorbtion
200
400
600
800
Wavelenght (nm)



UV-Vis spectrum of 10 μM CoQ1 recorded in kinetic mode in 1 M NaOH



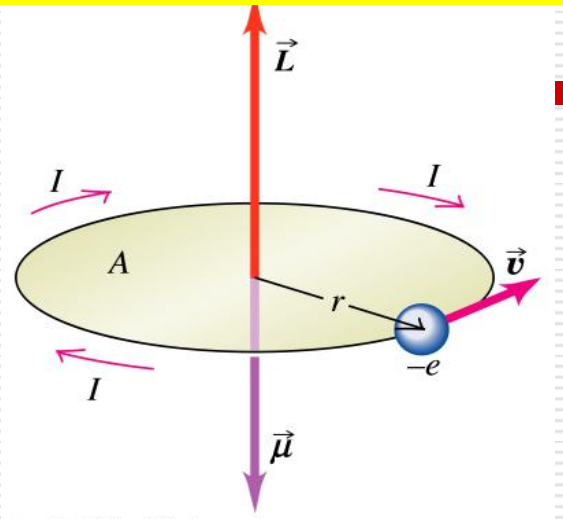
Comparison of the cyclic voltammograms of CoQ1 directly dissolved in pH of 7.00, and of CoQ1 initially dissolved in 1 M NaOH for 45 min and retitrated afterwards to pH of 7.00

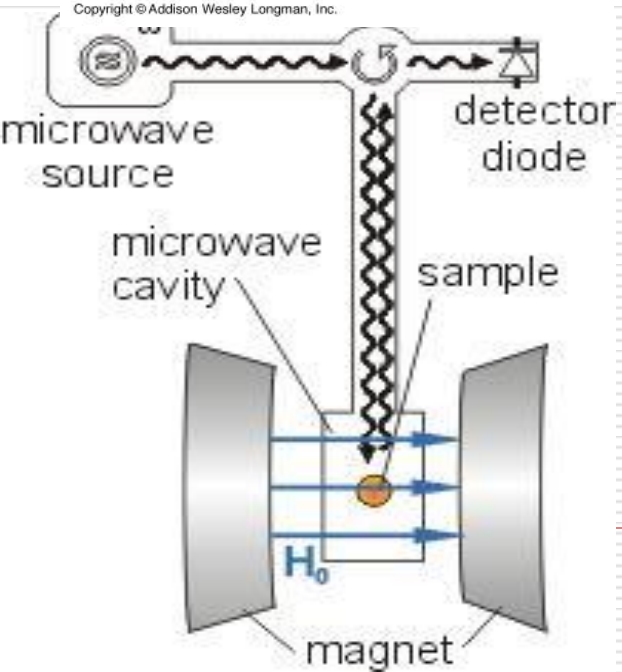


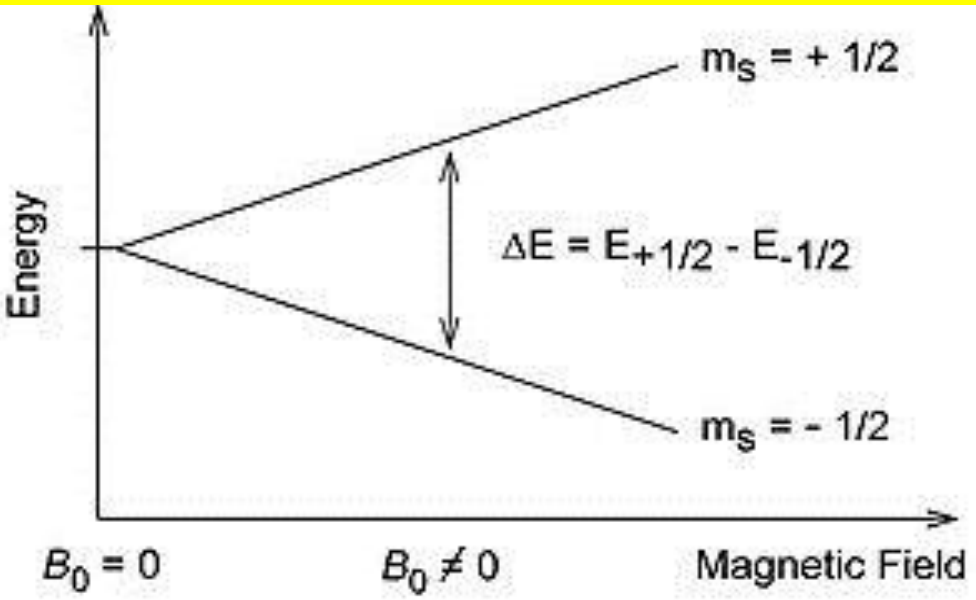
Effect of pH to the redox processes of CoQ1. In this situation CoQ1 was in contact with 0.1 M NaOH for 60 minutes

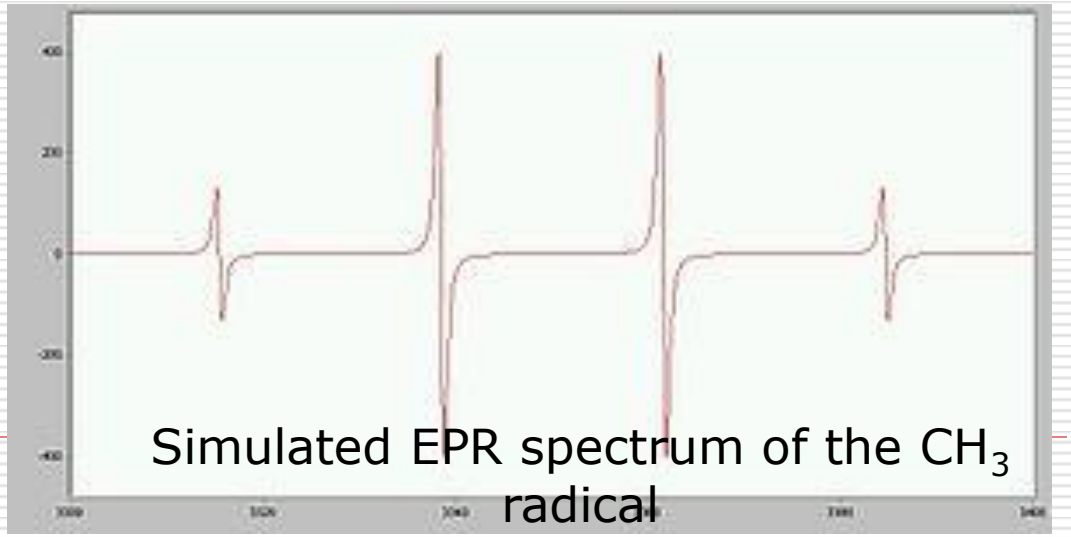
Next logical task: DETERMINE the MECHANISM and the STRUCTURE of the New Benzoquinone Product

Electron Paramagnetic resonance-EPR-suitable technique for structure evaluation by the radical species
(**many quinones form radicals when dissolved in alkaline media**)





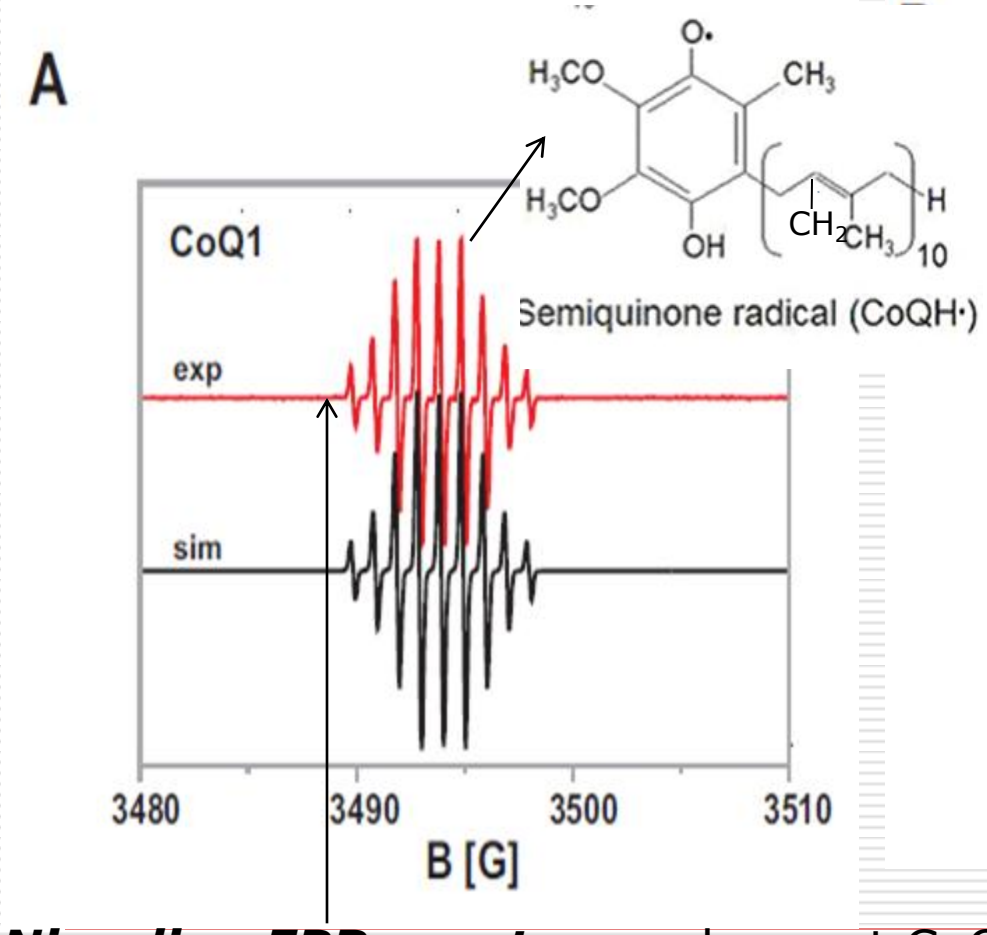


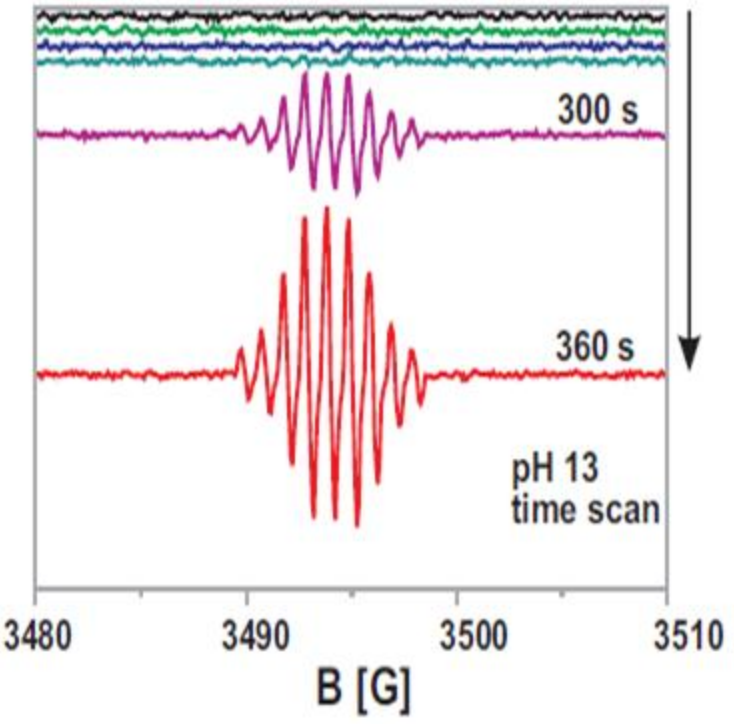


Simulated EPR spectrum of the CH_3 radical

EPR spectrum of CoQ1 after reduction with half-equimolar amount of $NaBH_4$ in pH of 7.00

EPR spectrum of CoQ1 obtained **in 0.1 M NaOH** but **without using any reductant**!!





Nine-line EPR spectrum od parent CoQ1 corresponds to **presence of one CH_3 and one CH_2 group** in the structure

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SPECTROCHIMICA ACTA PART A

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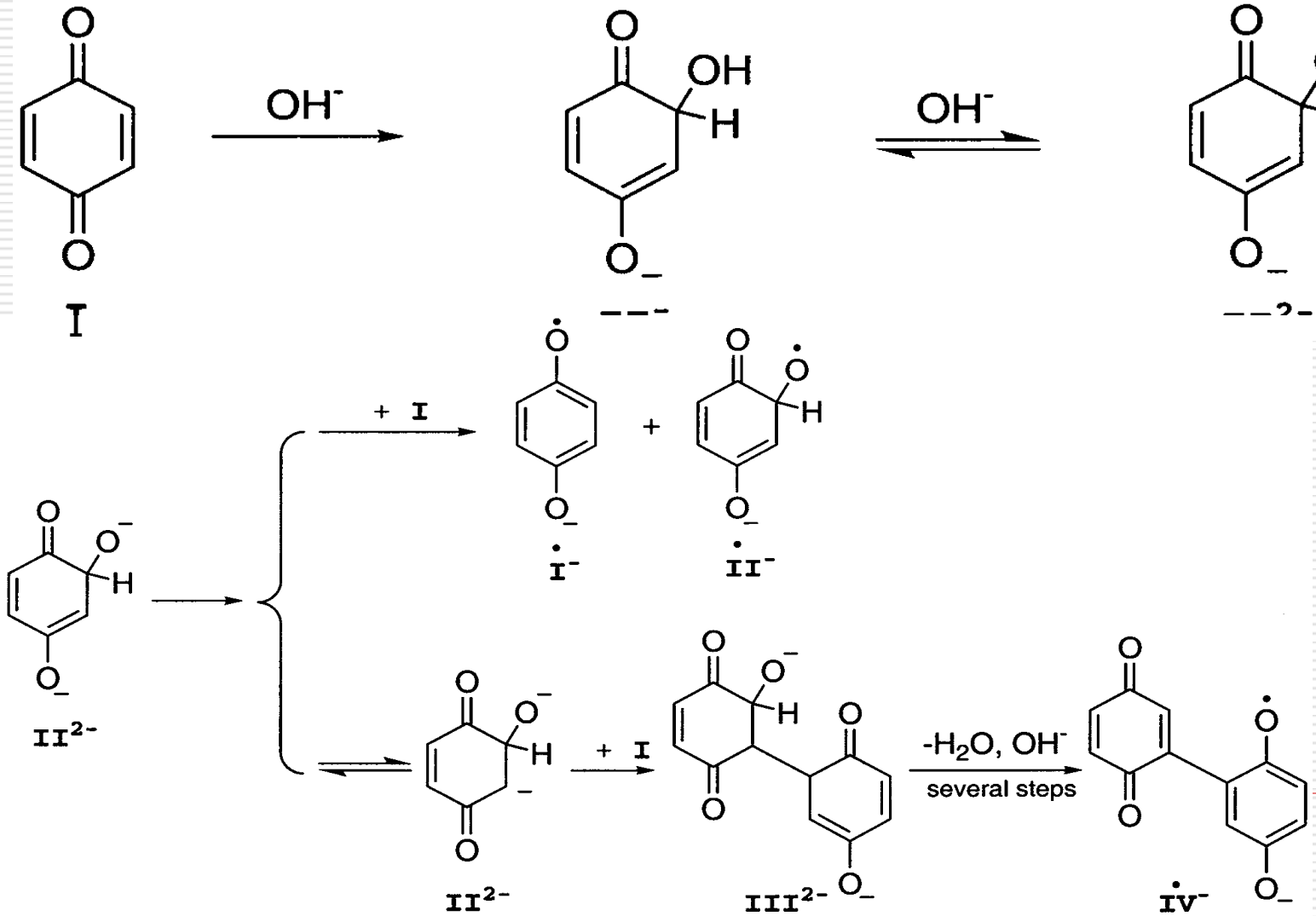
On the application of electron paramagnetic resonance in the study of naturally occurring quinones and quinols

Jens A. Pedersen



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Spectrochimica Acta Part A 58 (2002) 1257–1270

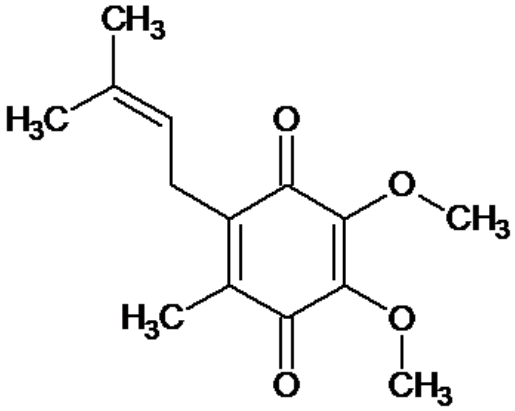
SPECTROCHIMICA ACTA PART A

www.elsevier.com/locate/saa

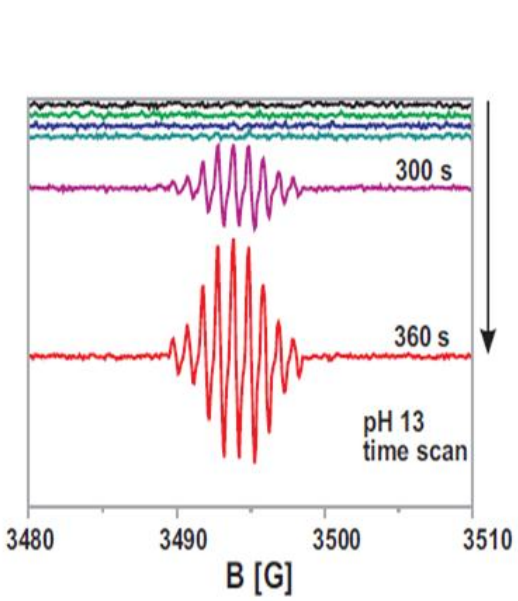
On the application of electron paramagnetic resonance in the study of naturally occurring quinones and quinols

Jens A. Pedersen

1. All anthraquinones and all 2,3-disubstituted naphthoquinones and tetrasubstituted benzoquinones [11] require a reducing agent, say, sodium dithionite in order to be reduced [12].
2. Benzo- and naphthoquinones with at least one unsub po dergo b (see b
3. Mixtu t u in cas e

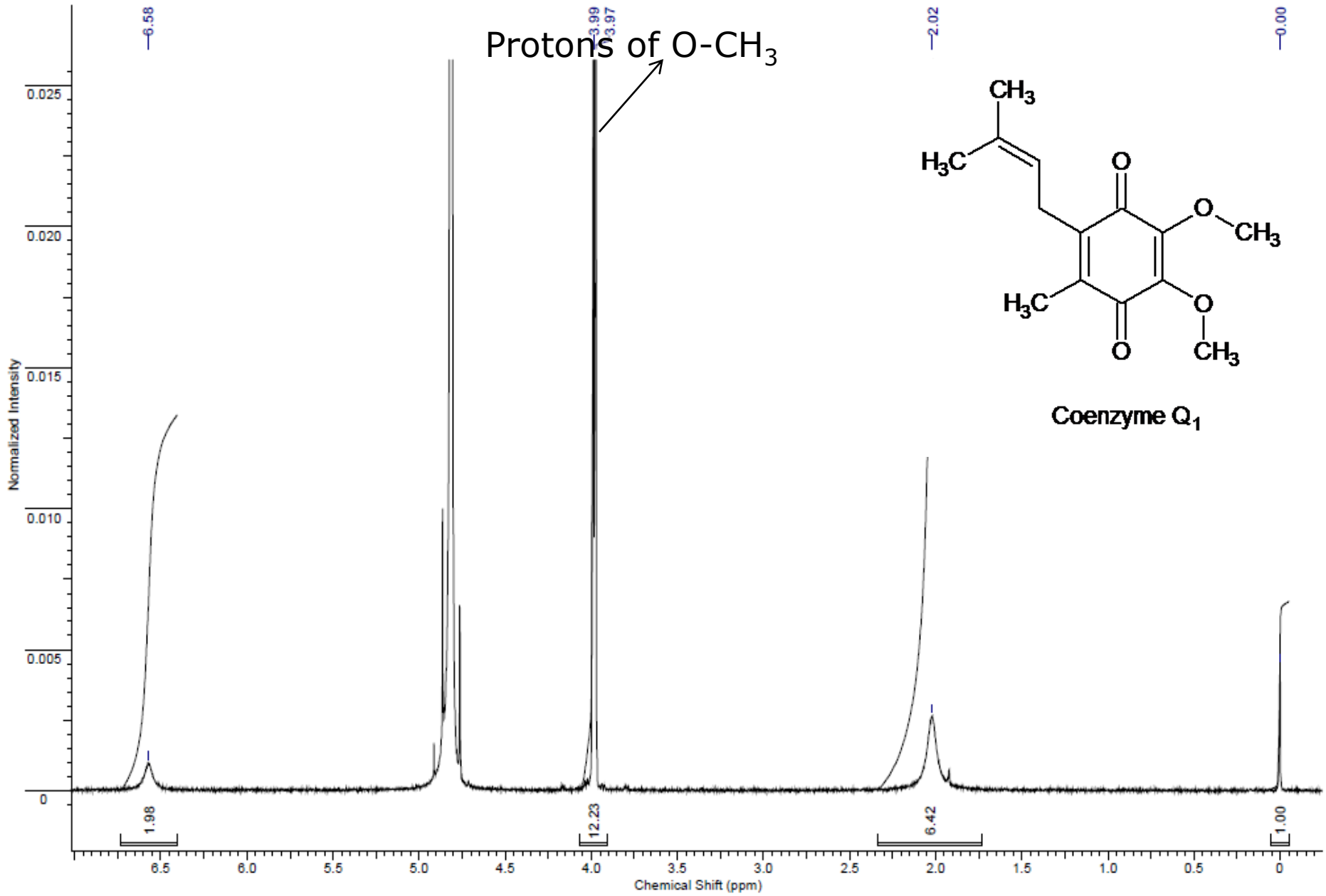


Coenzyme Q_1



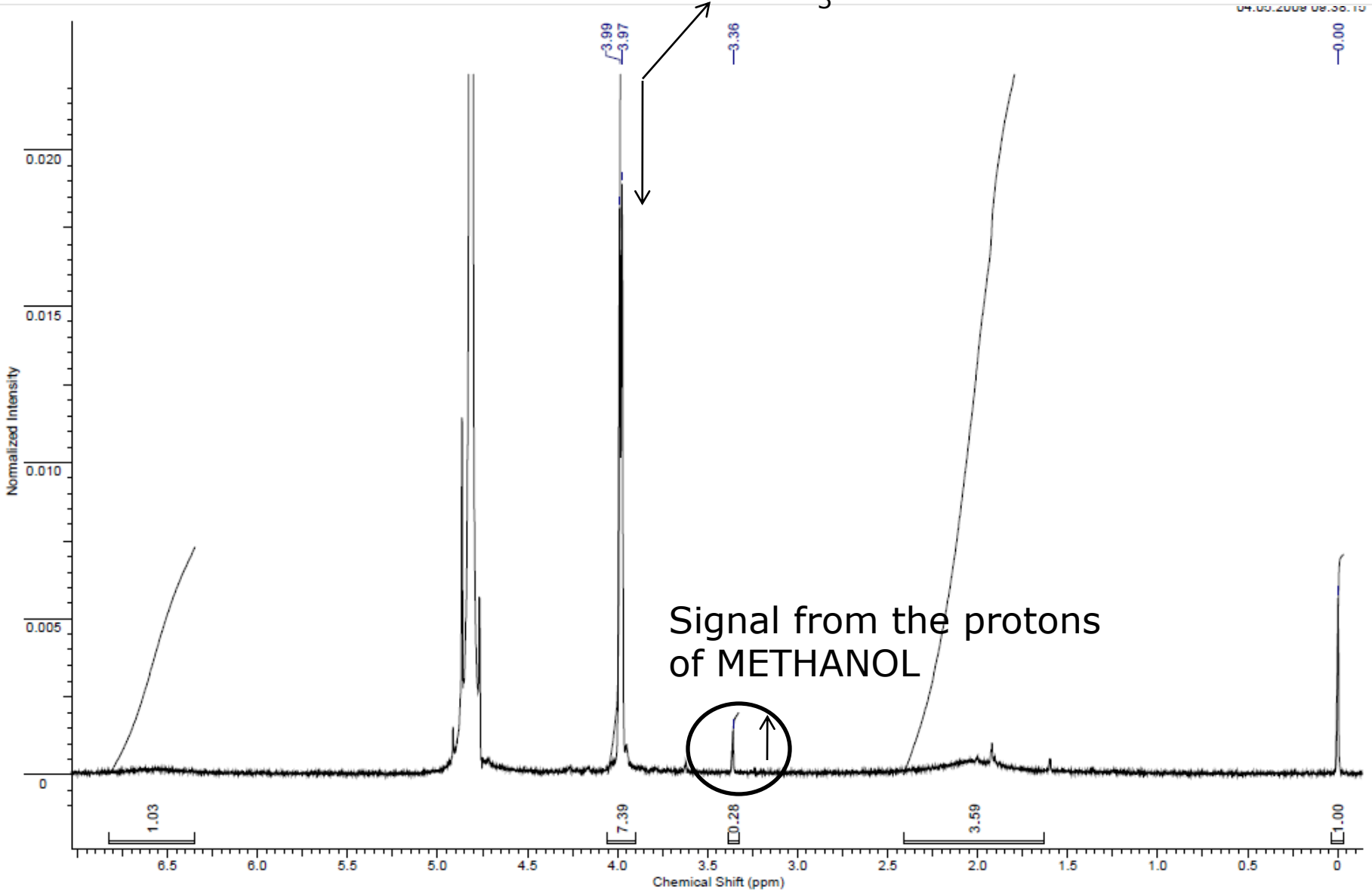
NEXT STEP to determine the structure-**NMR EXPERIMENTS**

NMR spectrum of CoQ1 in D_2O

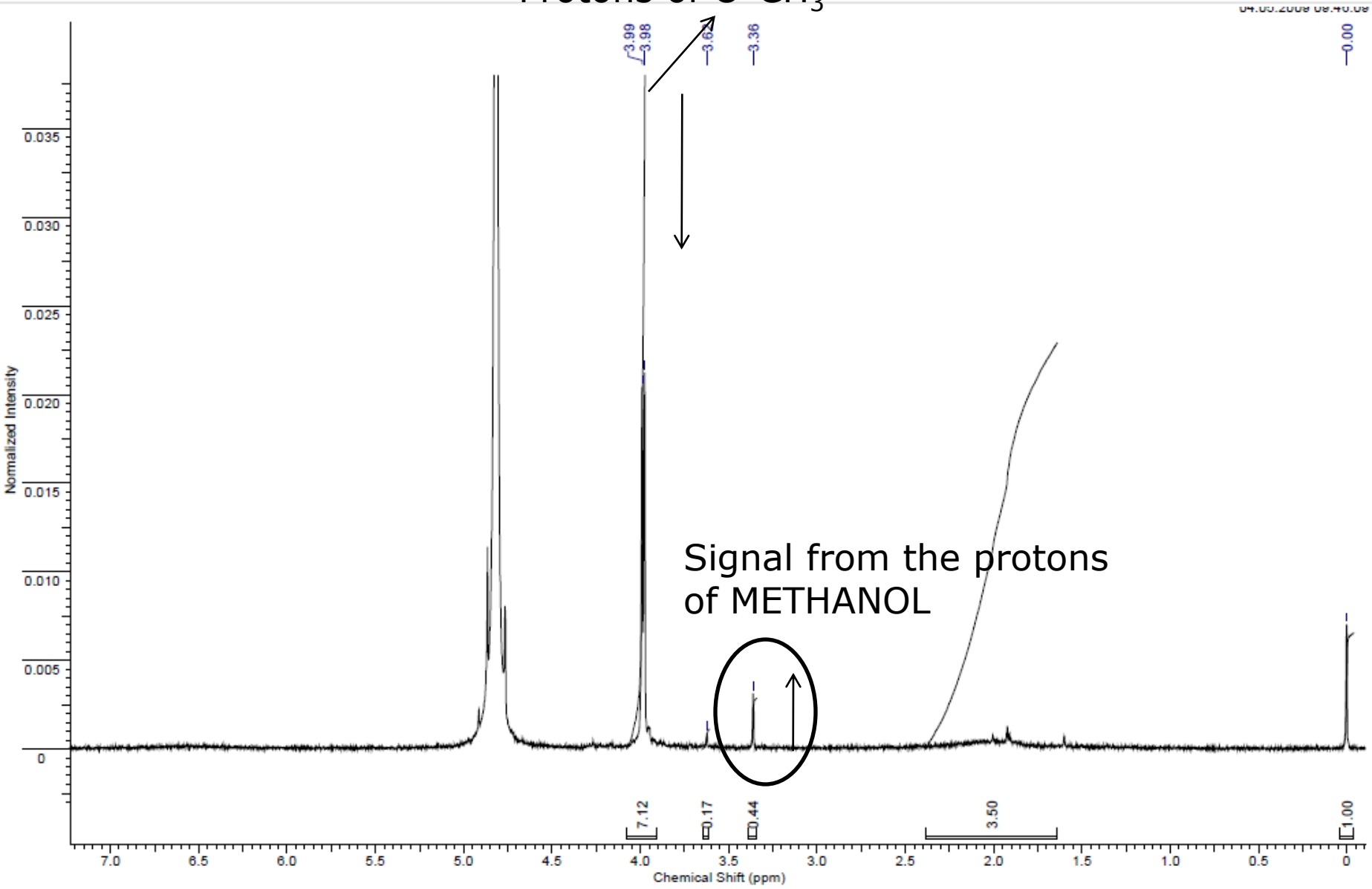


Coenzyme Q_1

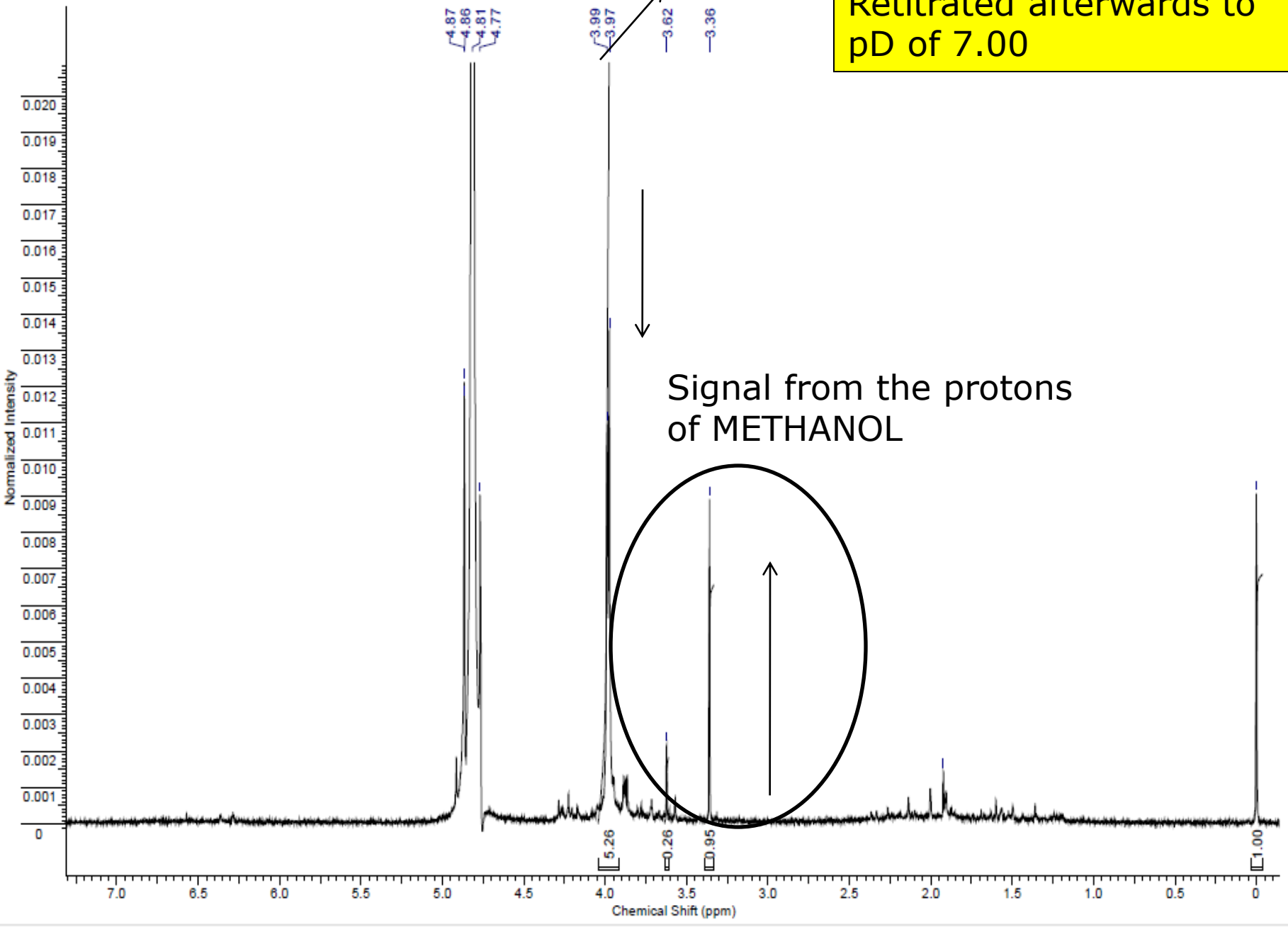
CoQ1-5 minutes in NaOD
retitrated afterwards to
pD of 7.00



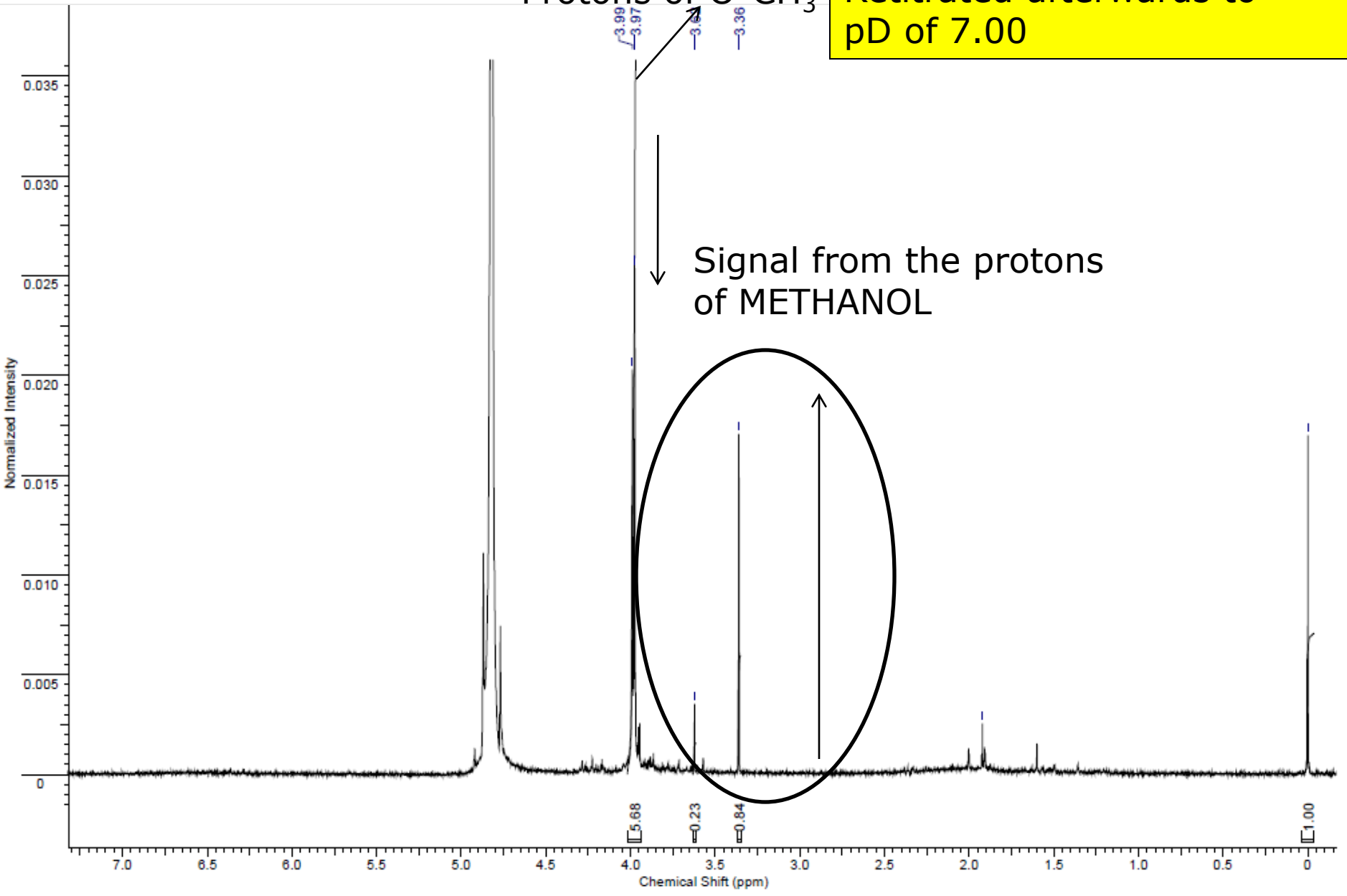
CoQ1-10 minutes in NaOD
Retitrated afterwards to
pD of 7.00

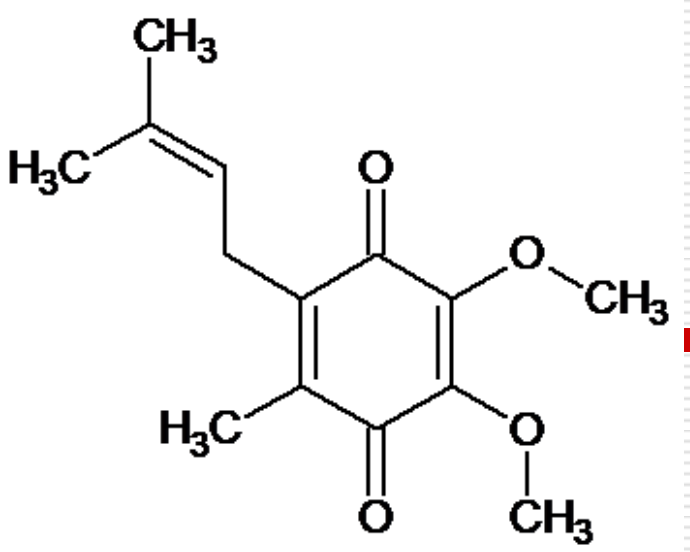


CoQ1-25 minutes in NaOD
Retitrated afterwards to
pD of 7.00



CoQ1-60 minutes in NaOD
Retitrated afterwards to pD of 7.00

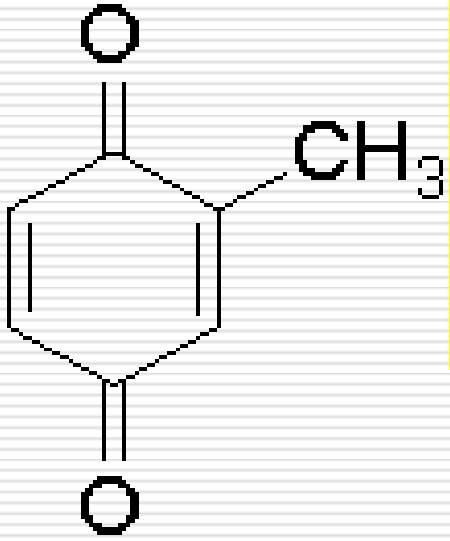




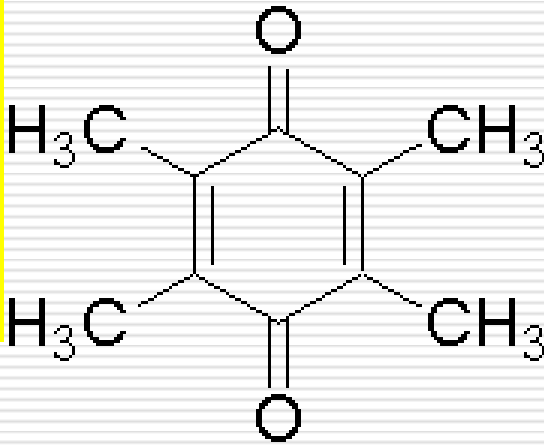
Coenzyme Q_1

Where *METHANOL* does come from, when CoQ is dissolved in alkaline media?
-from the *methyl group?*
or
-from the *METHOXY* O-CH_3 group?

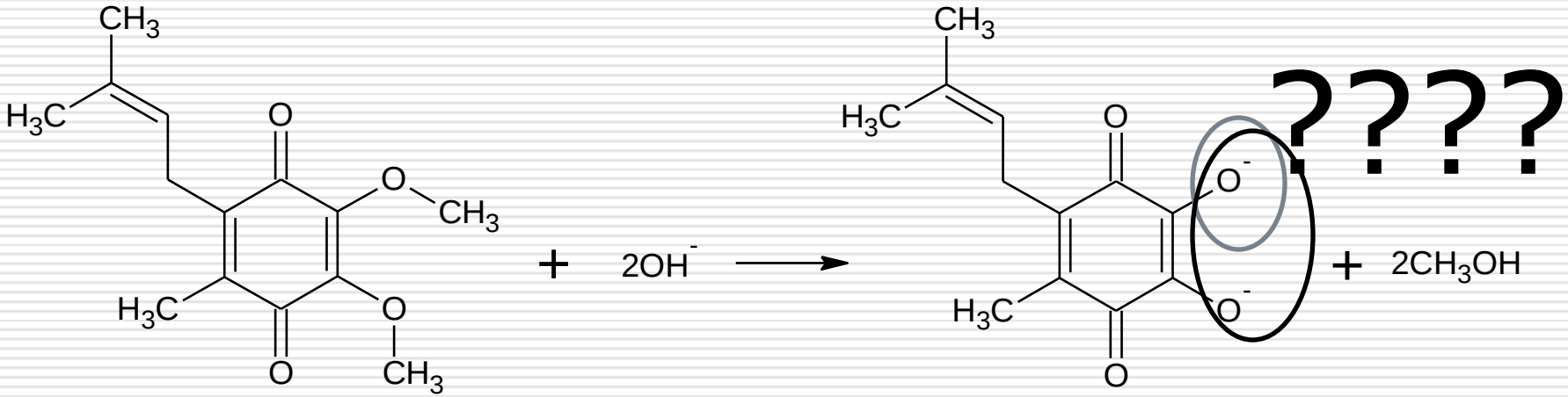
By using methyl benzoquinone derivatives we found that the *methyl group* CAN NOT BE CLEAVED from the aromatic ring!!!



2-methyl-benzoquinone



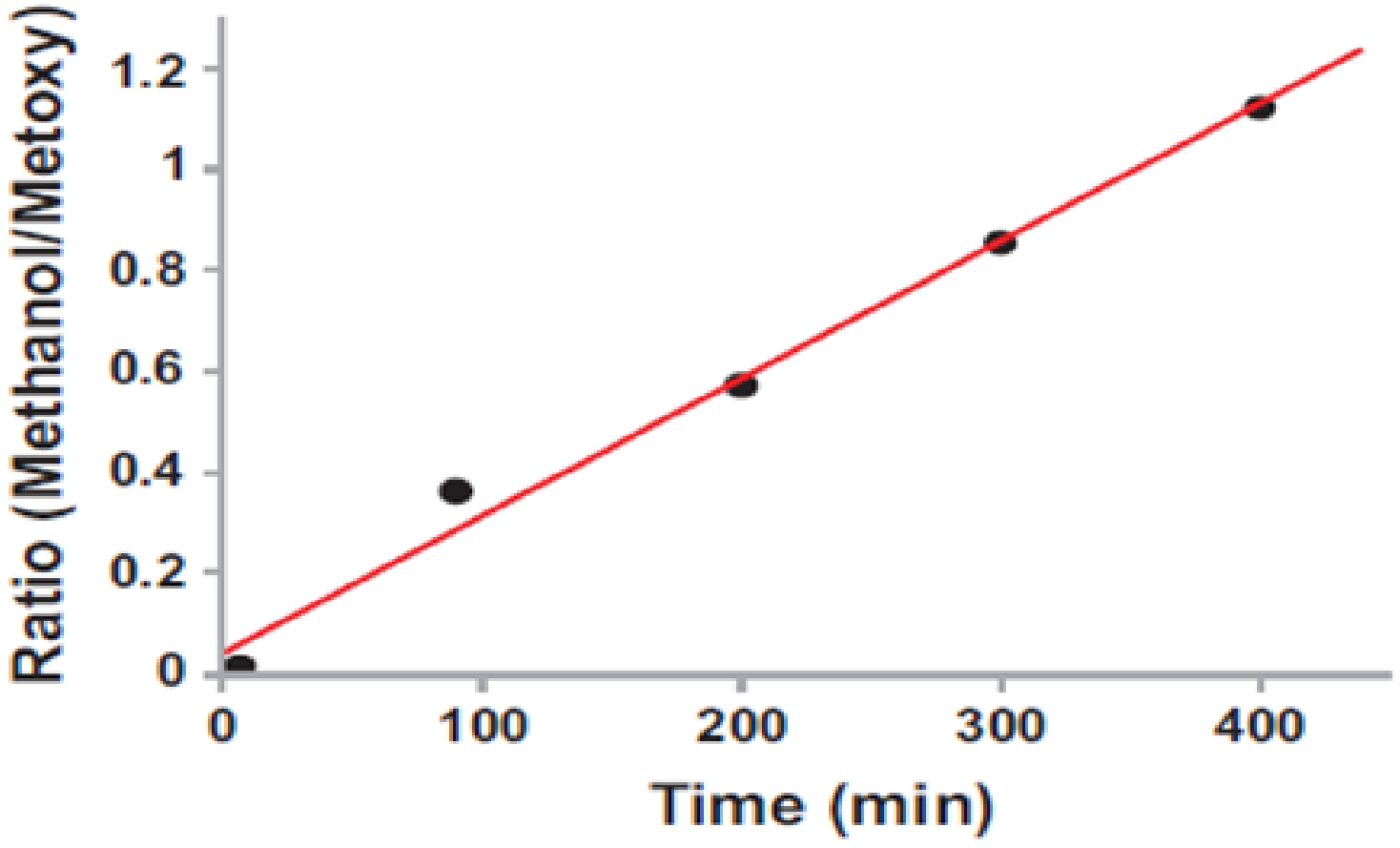
Tetramethyl benzoquinone (duroquinone)



Coenzyme Q_1

O-demethylated Coenzyme Q_1

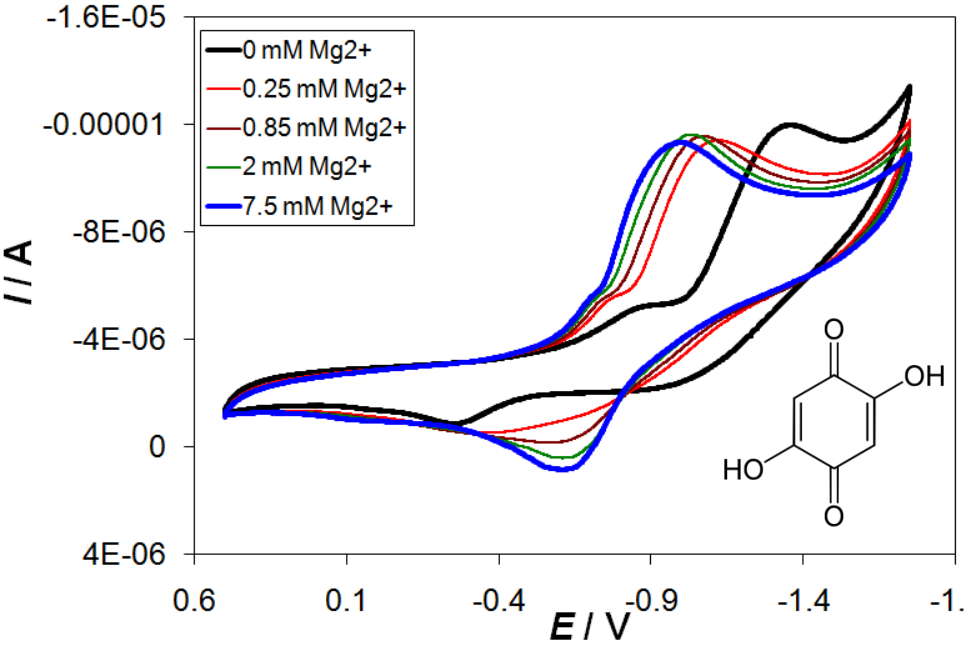
(2,3-**dihydroxo**-5-methyl-6-isoprenyl-benzoquinone)



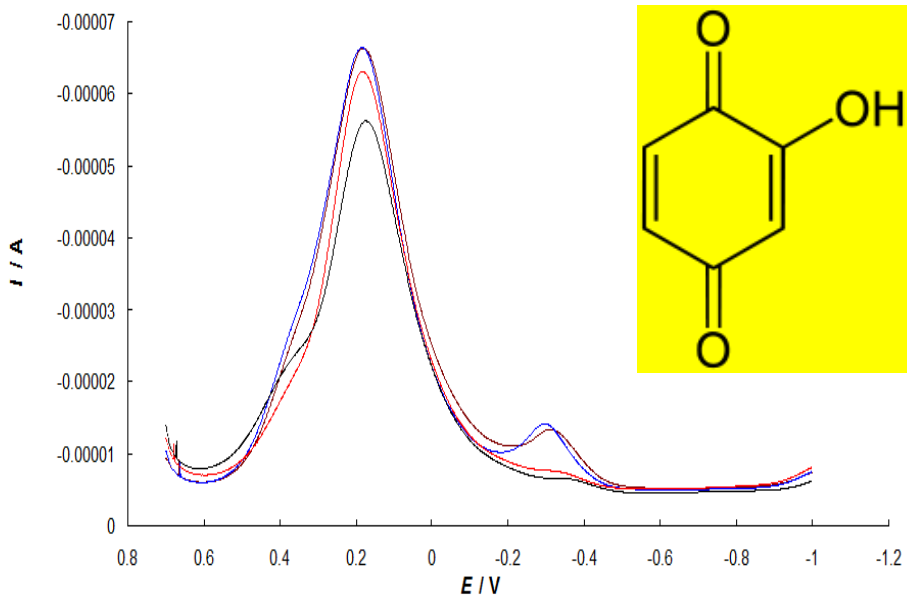
Ratio of the signal of Methanol vs Methoxy groups from NMR experiments of CoQ1 in NaOD

Color of solutions of
Benzoquinones with
2 OH groups in its structure

Color of solutions of
Benzoquinones with
1 OH group in its structure

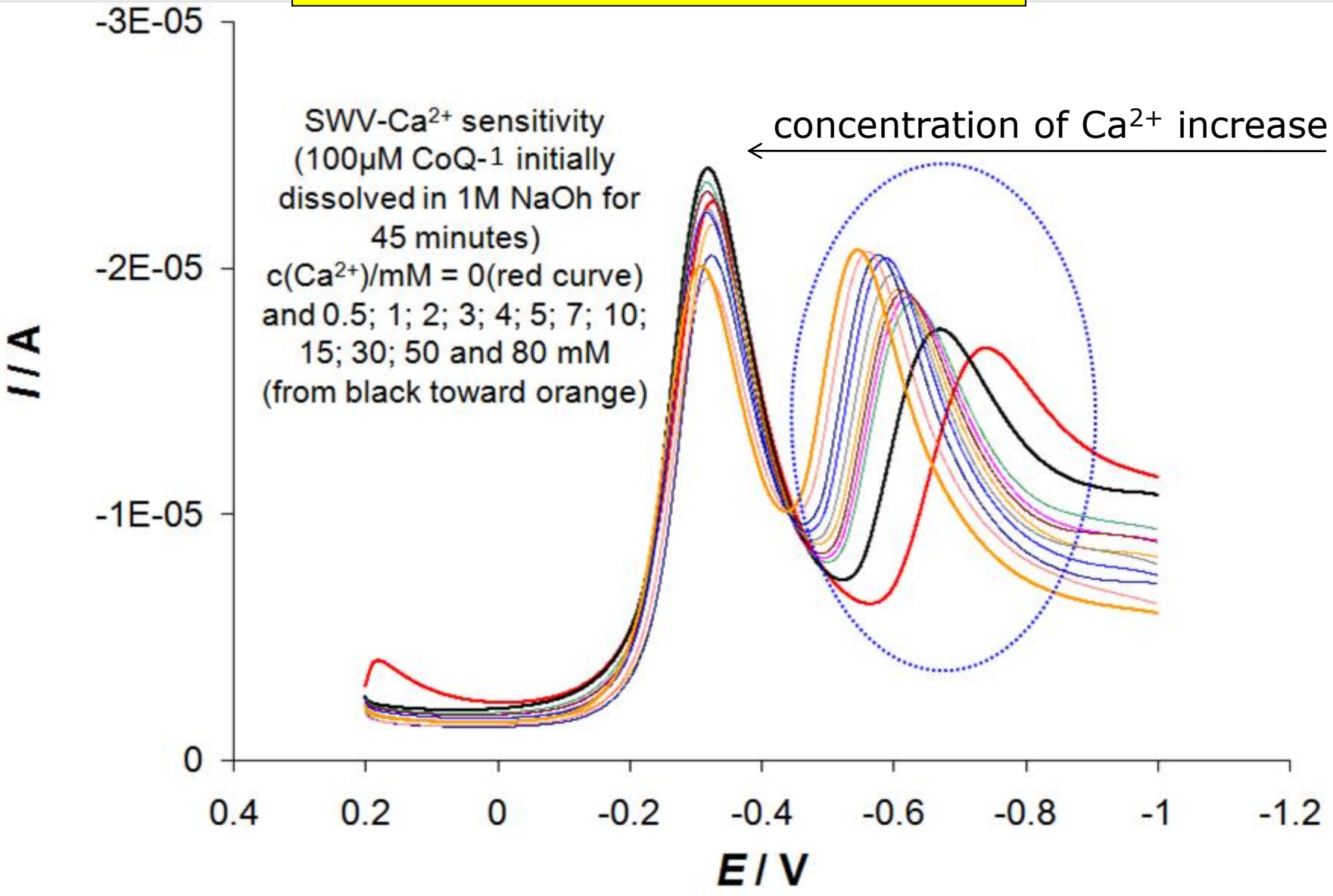


Benzoquinones with 2-OH groups are able to bind earth-alkaline cations in ratio 1:2

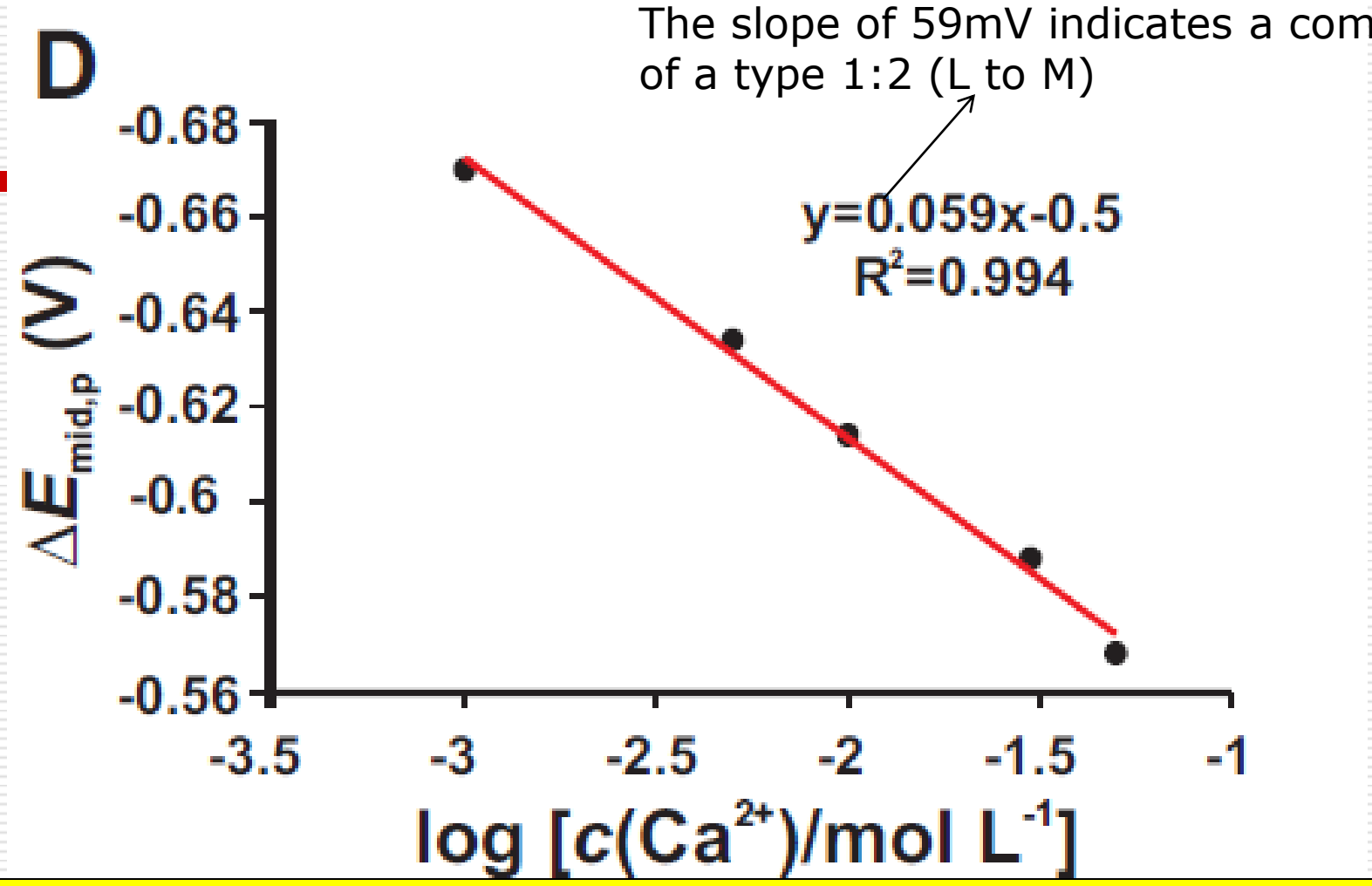


Benzoquinones with 1-OH group **are NOT able** to bind (at least not strongly) earth-alkaline cations

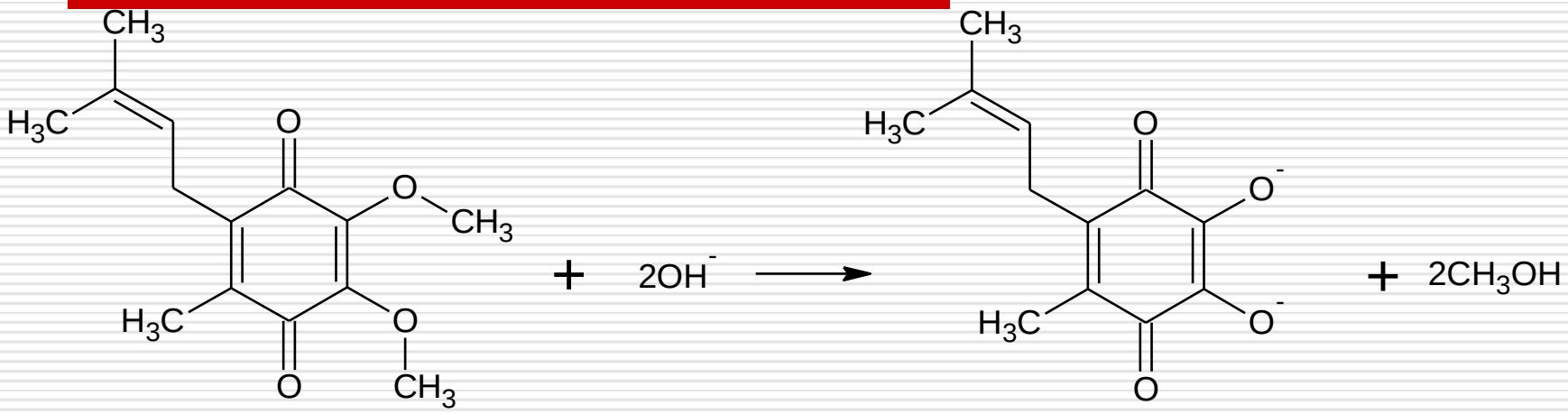
New product is able to bind Ca^{2+} ions in stochiometric ratio 1:2 ($L:M^{2+}$)



New form of CoQ-1, sensitivity to Ca^{2+} ions

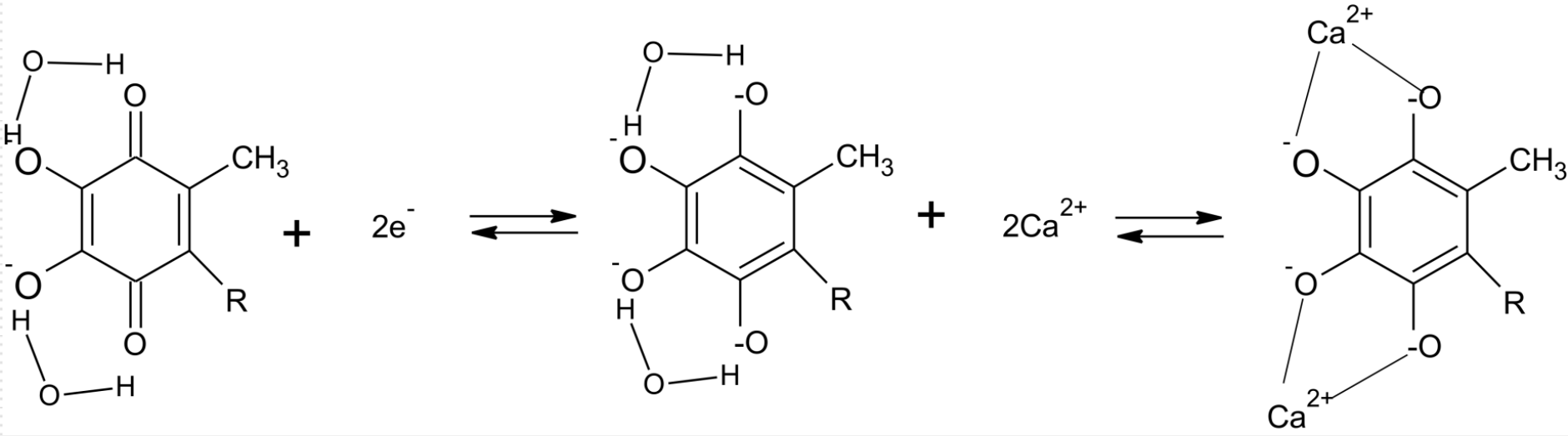


Dependence of the mid-peak potential of the cyclic voltammograms of new form of CoQ1 on the logarithm of Ca^{2+} concentration

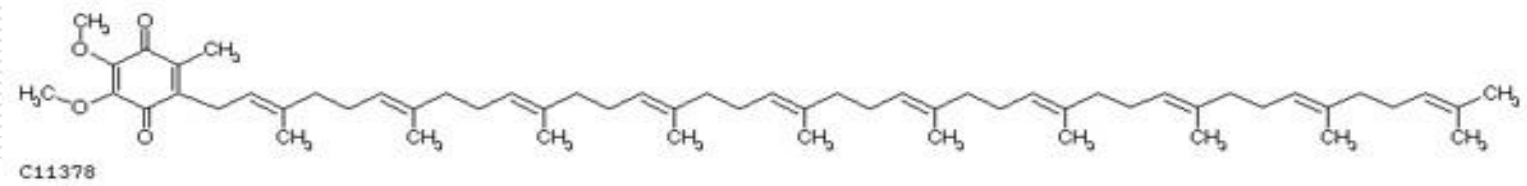


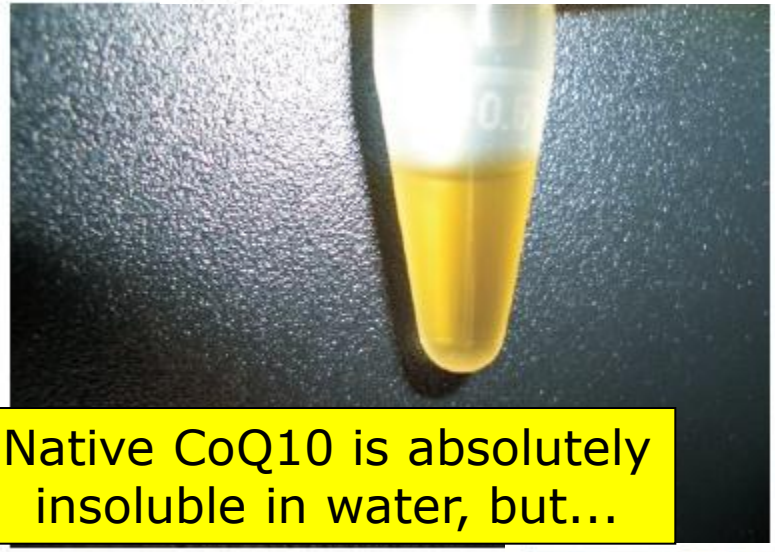
Coenzyme Q_1

O-demethylated Coenzyme Q_1

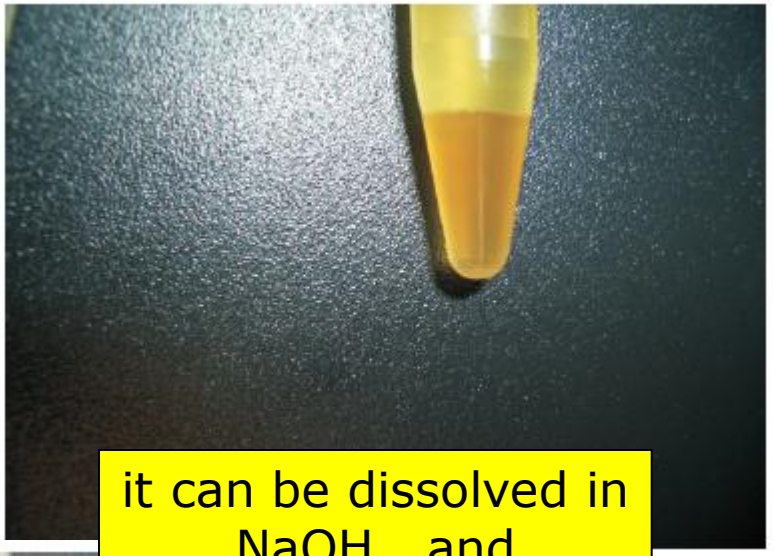


EXPERIMENTS with Coenzyme Q10-CoQ10

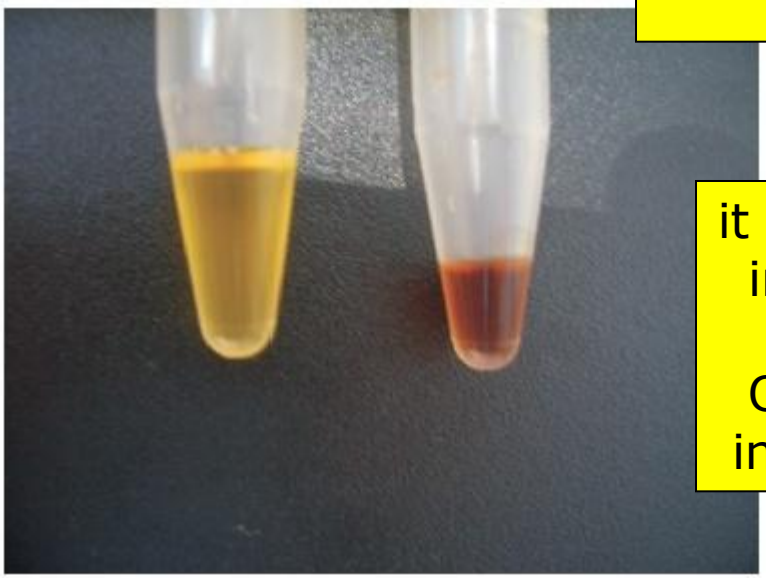




Native CoQ10 is absolutely insoluble in water, but...

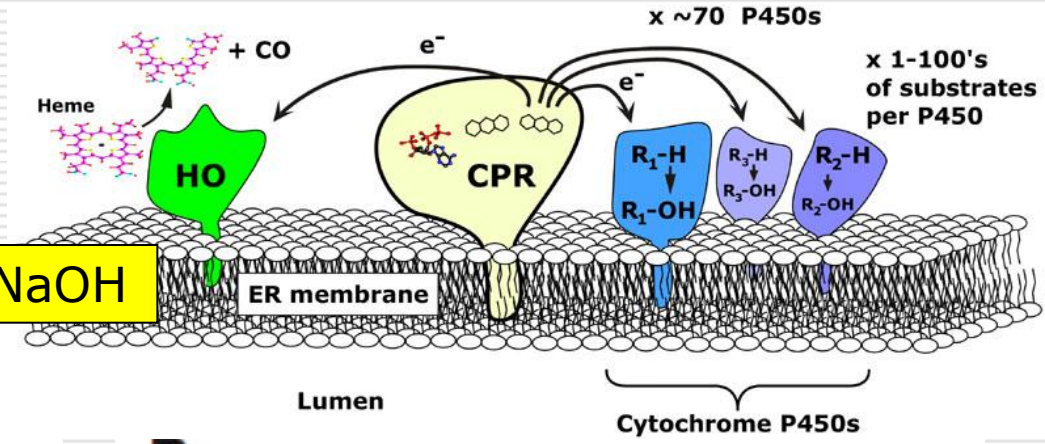


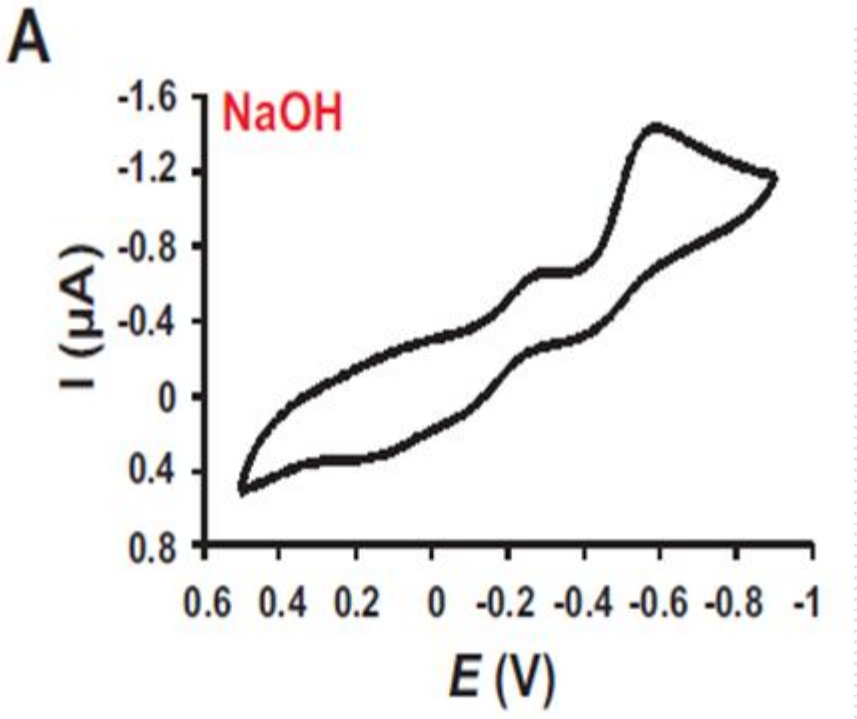
it can be dissolved in NaOH...and

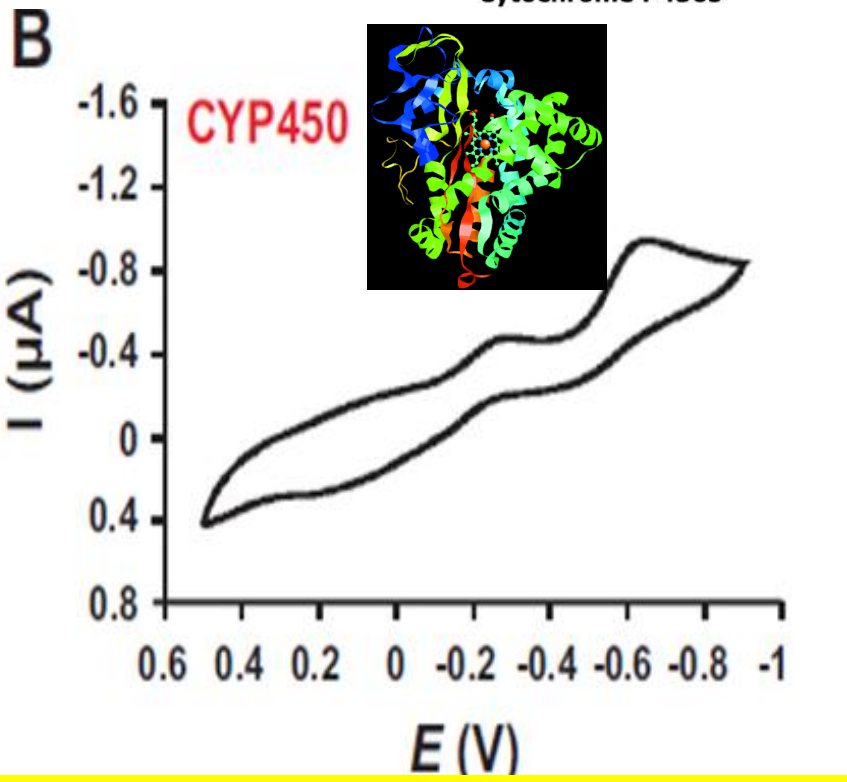


it can be transformed into a new form in presence of OH^- anions present in the organic phase

Coenyzme Q10 in presence of 1 M NaOH

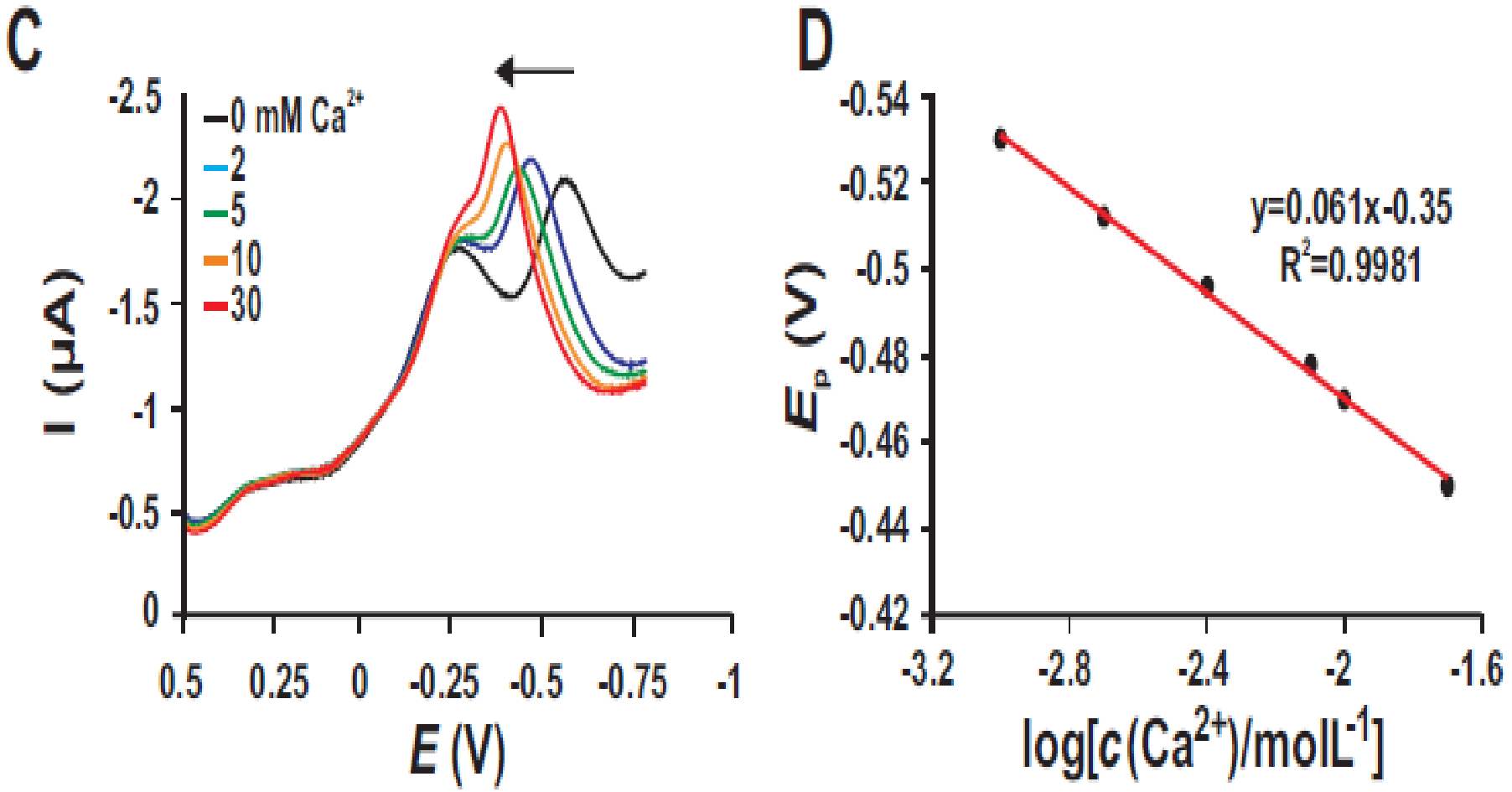




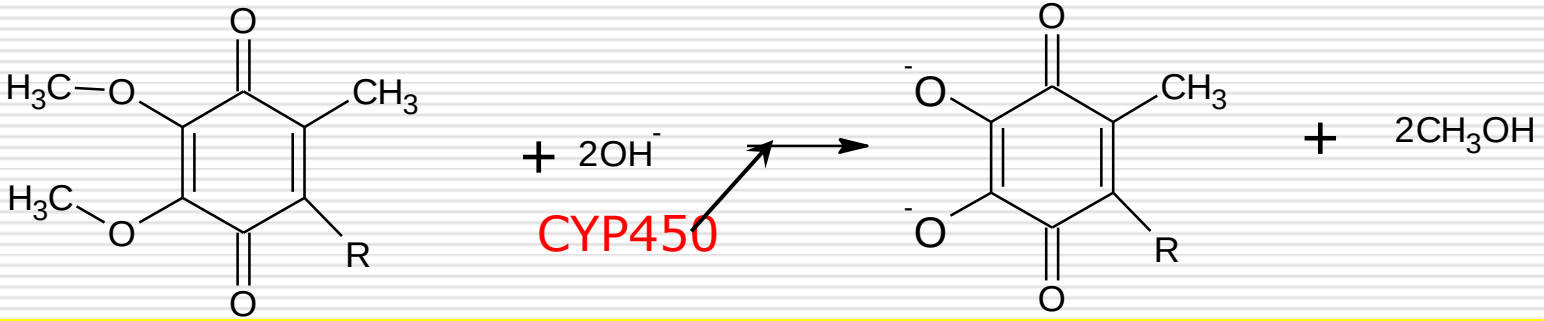


5 μM Coenyzme Q10 in presence of 1 M NaOH and retitrated to pH of 7.0 after 3 weeks

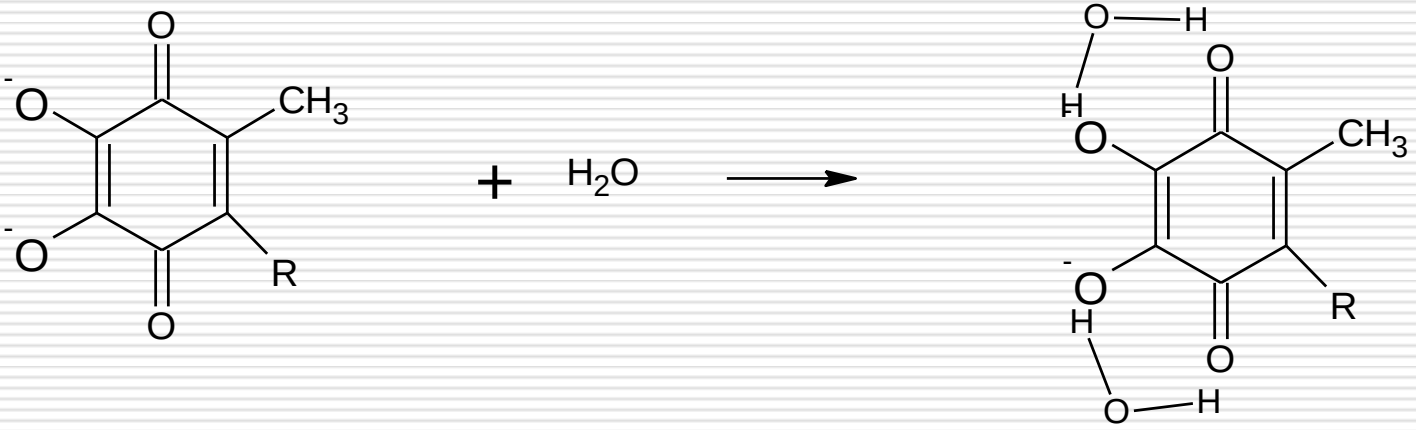
The membrane-bound enzyme Cytochrome P450 does the same task as NaOH to CoQ10!!!



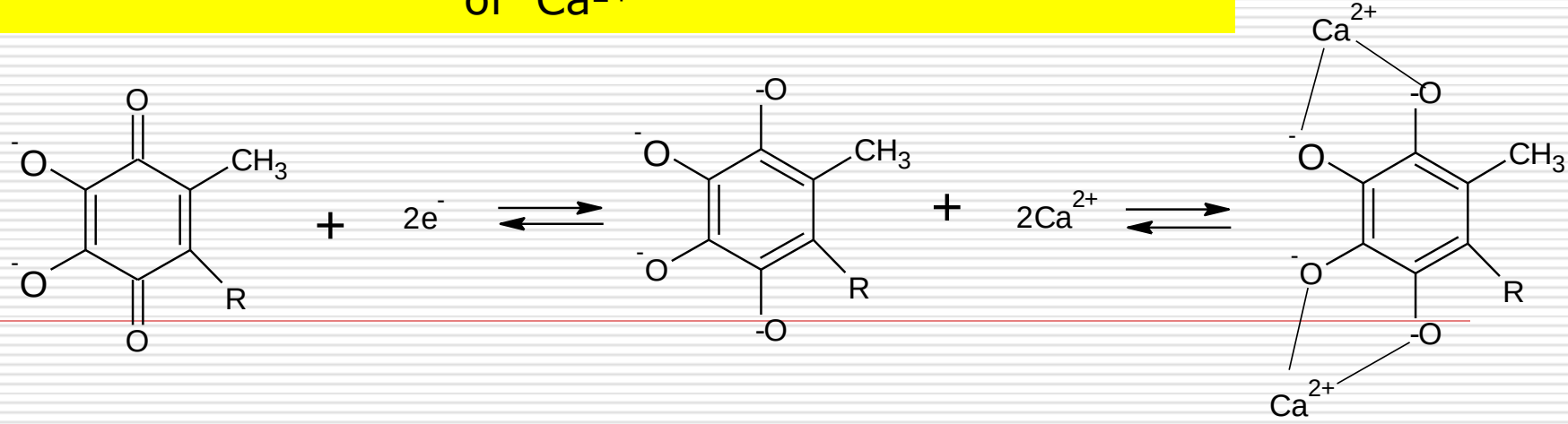
Effect of Ca^{2+} to the voltammetric response of CoQ10 in presence of CYP450 in pH of 7.40



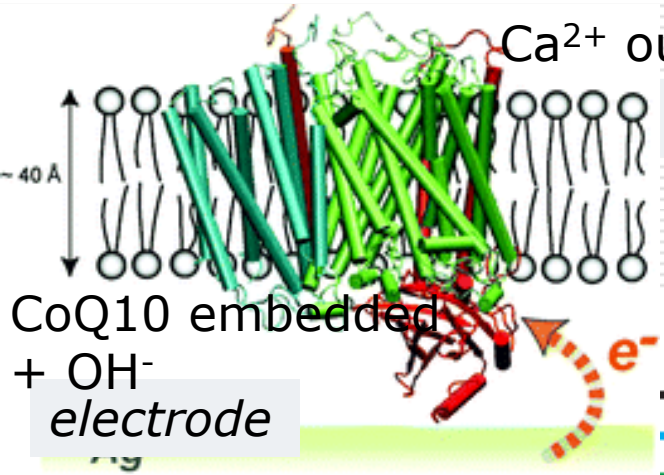
2. Stabilization of the CH_3-cleaved CoQ10 products in water

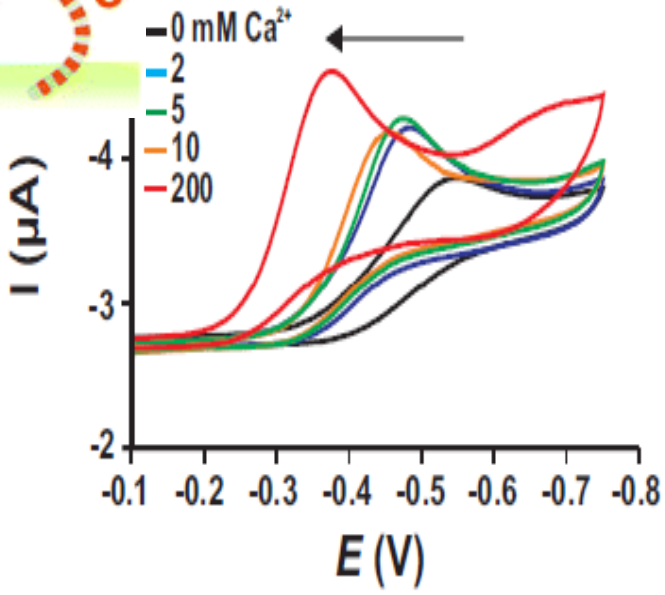


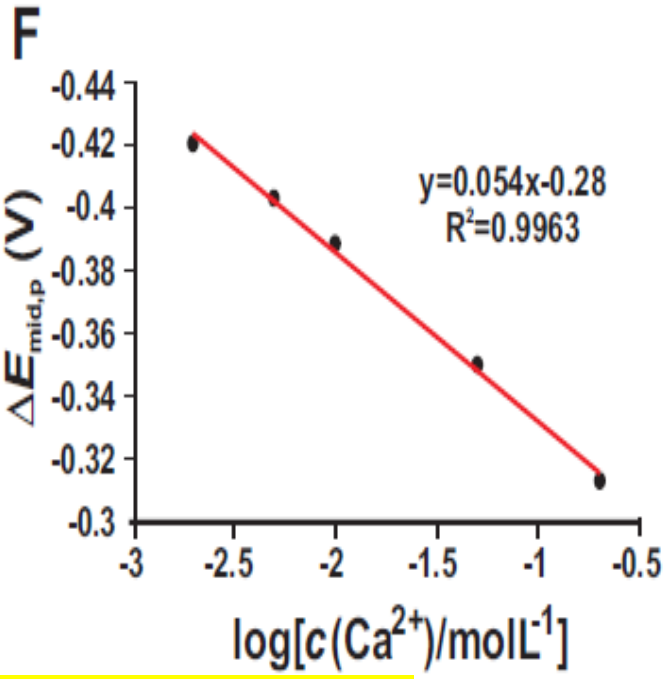
3. Reduction of the CH_3-cleaved CoQ10 product and binding of Ca^{2+}



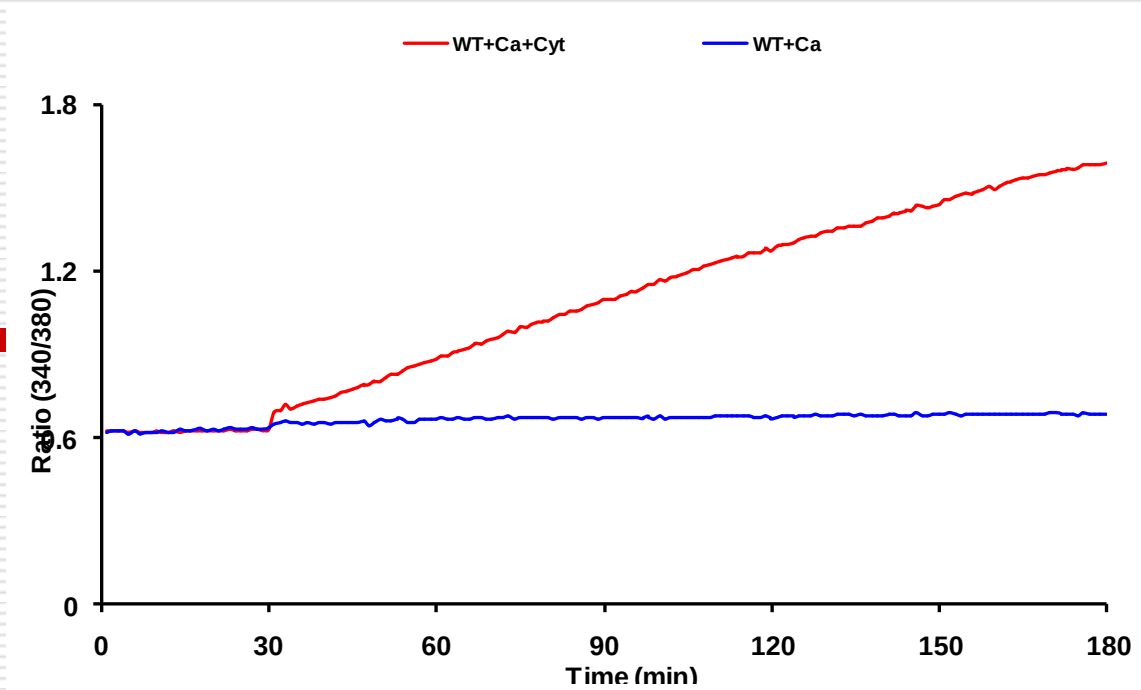
Experiments with CoQ10 embedded in organic membrane in presence of Organic Hydroxide to show whether the new form of CoQ10 can transfer Ca^{2+} ions across biological membranes

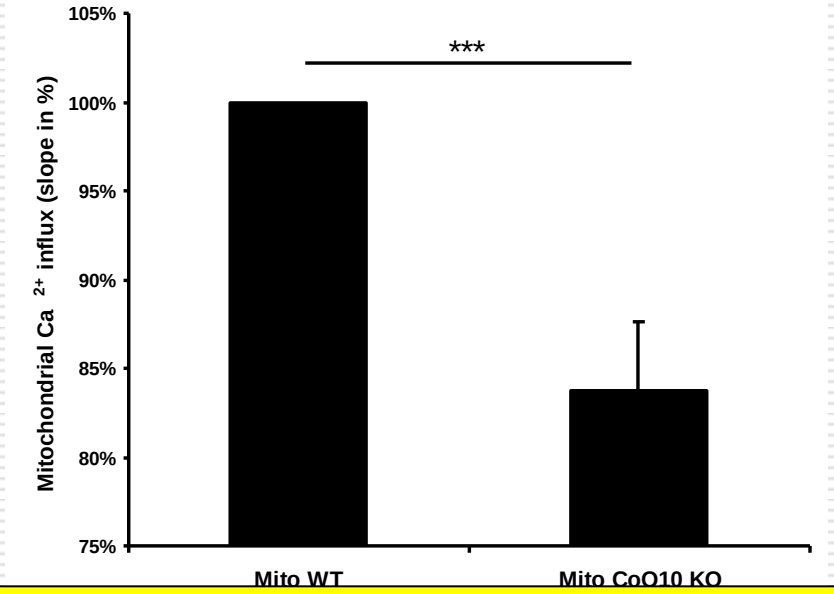




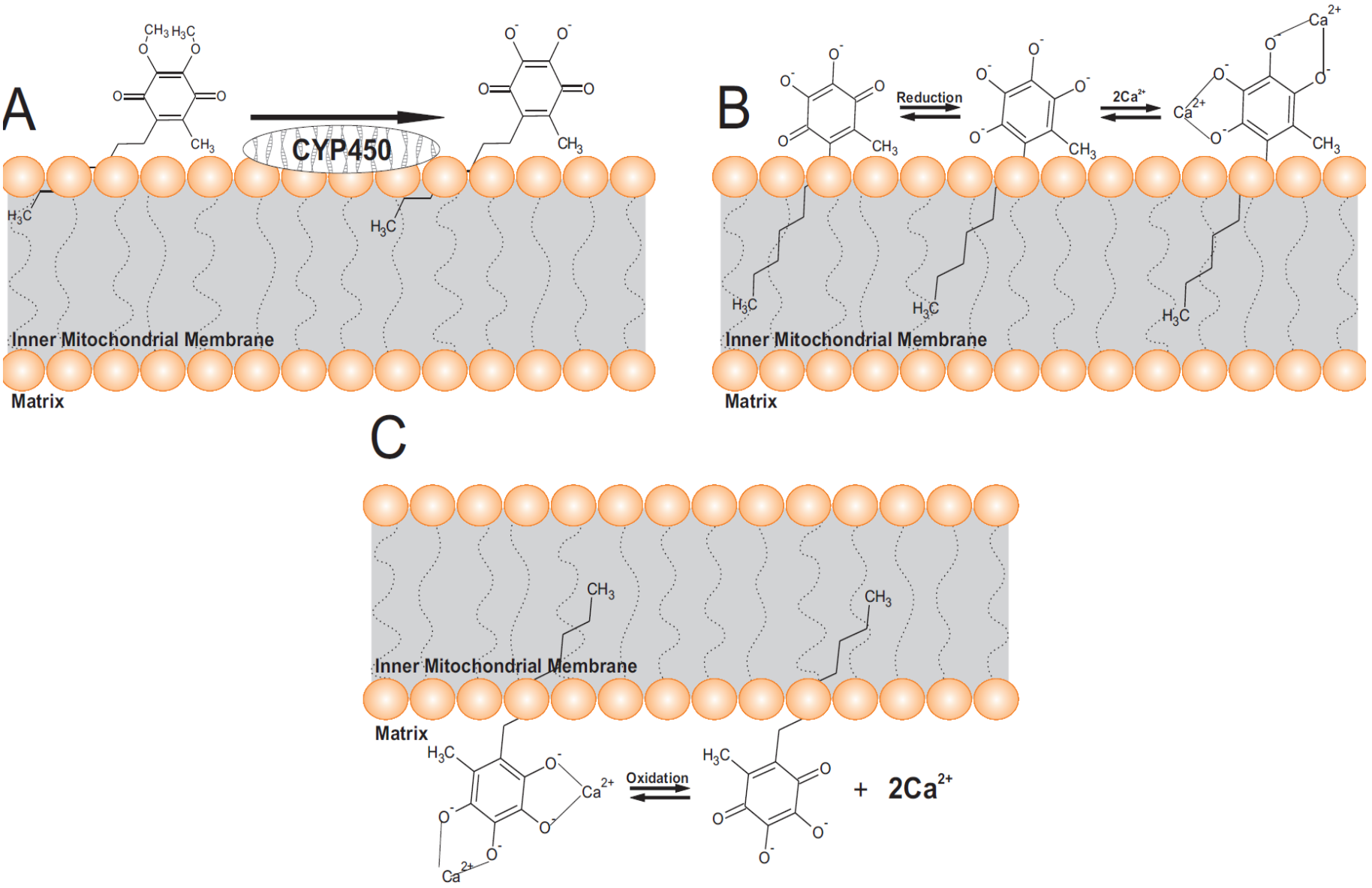


Cyclic voltammograms of CoQ10 showing transfer of Ca^{2+} ions across biomimetic membranes

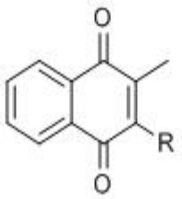


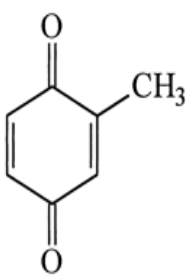


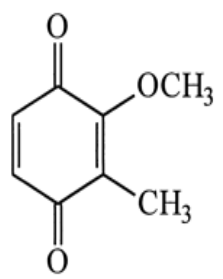
Experiments with wild types of mitochondria and knockout mitochondria depleted of CoQ10 in absence and in presence of CYTP450. Effect of Ca^{2+} ions


A
CYP450
Inner Mitochondrial Membrane
Matrix
B
Reduction
2Ca2+
Inner Mitochondrial Membrane
Matrix
C
Inner Mitochondrial Membrane
Matrix
Oxidation
+ 2Ca2+

SUMMARY



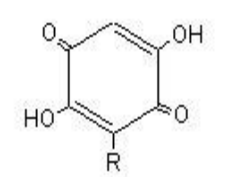




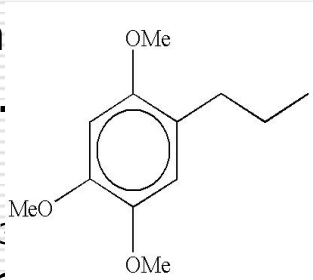
❑***Plenty of CoQ family members are prese… … …e li***

❑The chemistry and most of the functions of the native f
members are mainly portrayed in the features of the 2e-/
and proton transfer) that leads to reversible t tion of the quinone to quinol
forms.

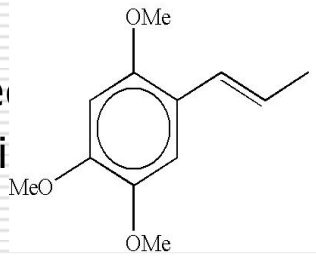
1 2



❑If the Coezyme Q structures are in contact with high concen
CYP450 enzymes, quite different quinonic forms can be obtained.

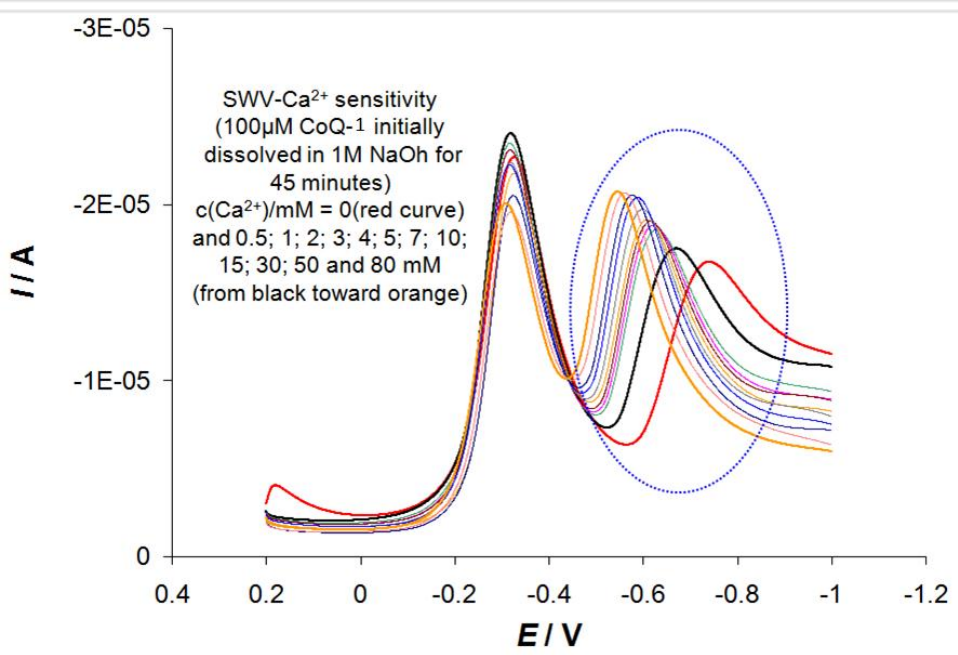


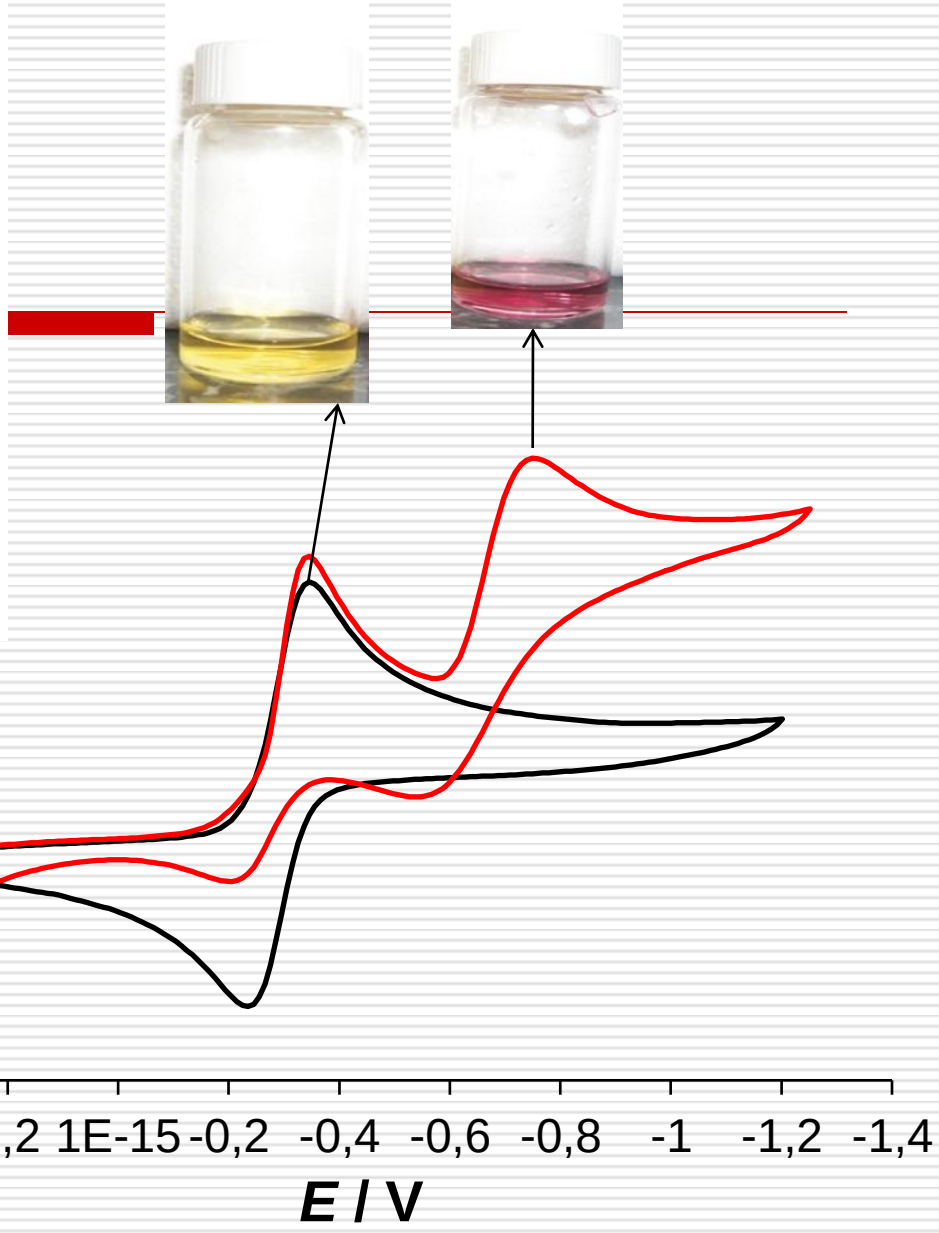
❑CYP450 and NaOH can both induce scission of the both O-CH
the structure of the Coenzyme Q family members, thus cr
demethylated" quinones that bear charge of „2-".



❑***These new Coenzyme Q structures*** formed in alkaline me
CYP450) are more polar than their parent compounds, whi
stronger antioxidative features.

❑The inherent properties of the new Coenzyme Q structures to bind the earth-alkaline cations upon their reduction classify these compounds as potential facilitators for transferring of metal ions across biological membranes.


SWV-Ca2+ sensitivity
(100µM CoQ-1 initially
dissolved in 1M NaOh for
45 minutes)
c(Ca2+)/mM = 0(red curve)
and 0.5; 1; 2; 3; 4; 5; 7; 10;
15; 30; 50 and 80 mM
(from black toward orange)
-3E-05
-2E-05
-1E-05
0
I / A
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1
-1.2
E / V


I / A
-1E-05
0
1E-05
0,6
0,4
0,2
1E-15
-0,2
-0,4
-0,6
-0,8
-1
-1,2
-1,4
E / V

Main people involved in this project

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Bernd Morgenstern

Barbara Kutzky

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Prof. Hermann

D: MKD

Prof. Mirceski

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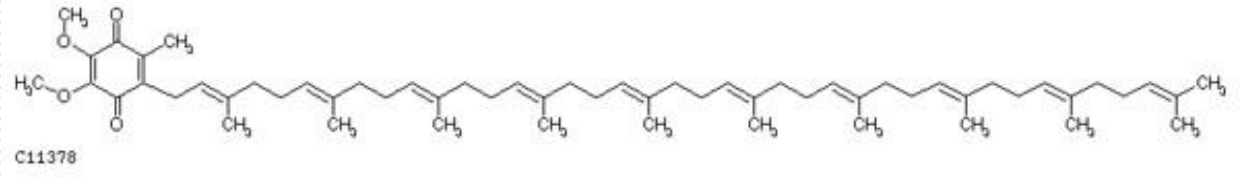
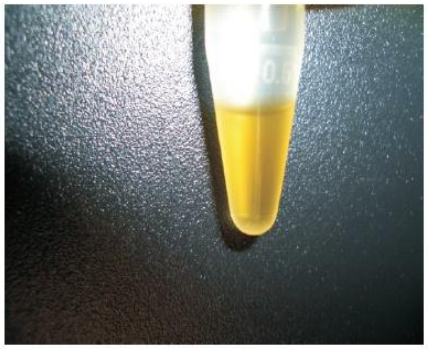
Alexander von Humboldt
Stiftung/Foundation

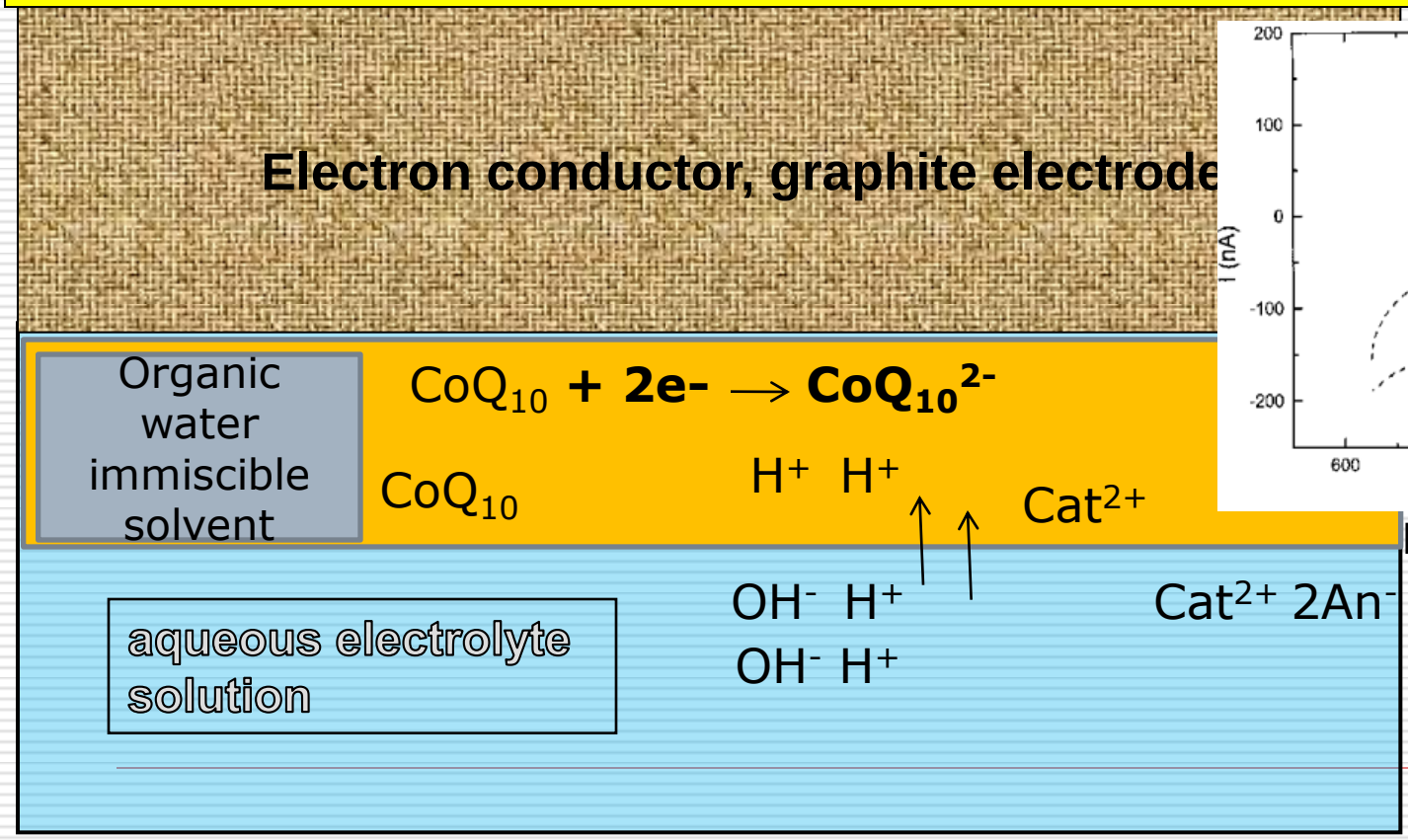
Experiments with Coenzyme Q10-CoQ10

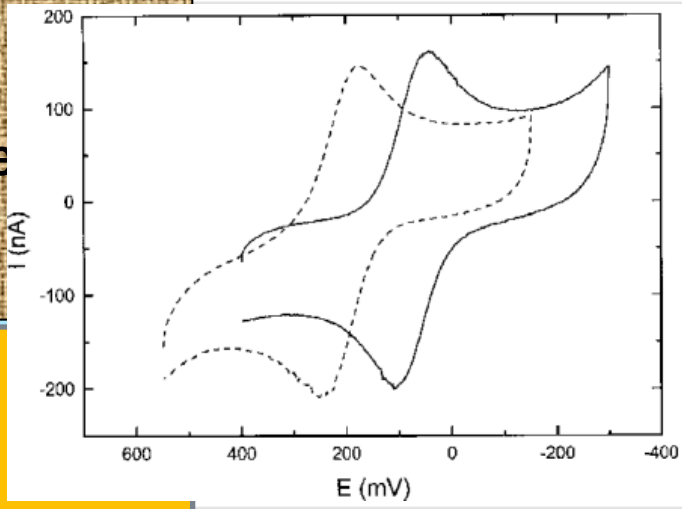
Solubility of CoQ10 in water is bellow 10 pM!!!
Impossible to perform experiments in water media!



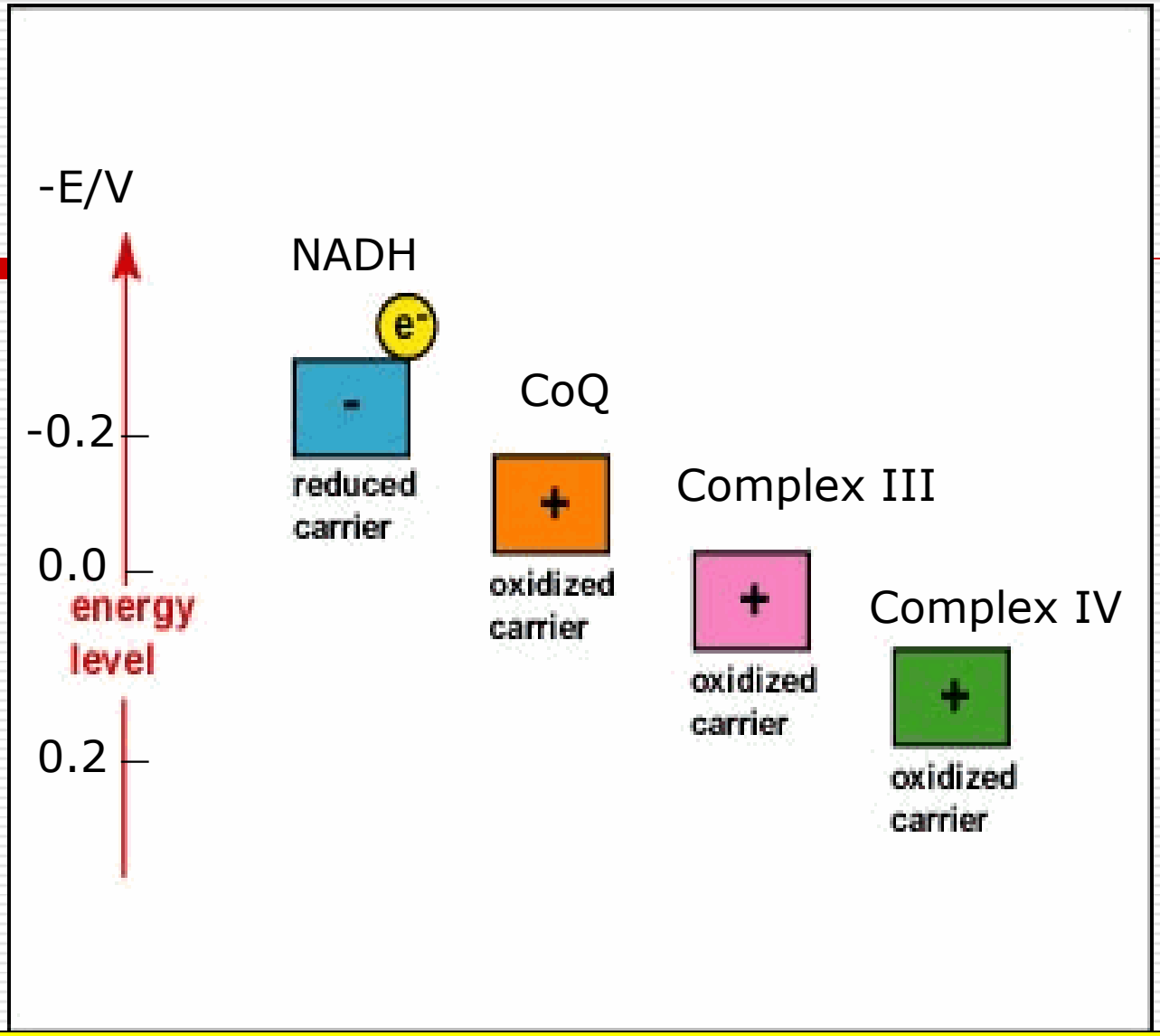


CoQ10 studied voltammetrically in **Thin-film voltammetry set-up**





Liquid-Liquid interface



The processes in the mitochondrial electron transfer chain are driven by the differences in the standard redox potentials of the contributors