„Reactive oxygen species and antioxidants: Implication for skin aging”

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Questions:

Radicals or reactive species, oxygen and nitrogen?

How these species are formed?

How to measure these species?

Influence of external stress

Reactive species and antioxidants

Reactive species and skin

Reactive species and aging

What are reactive oxygen species?

- Radicals or something else?

Radicals - Definition

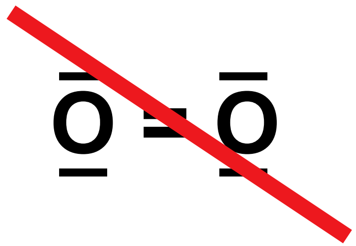
- Chemical species with unpaired electrons
  e. g.: H• or NO•, and O_2 .
- An unpaired electron has a magnetic moment and a spin quantum number.
- Radicals have 2 different orientations in a magnetic field → doublet state.
- Biradicals like oxygen have 3 different orientations in a magnetic field → triplet state.
- Radicals are not necessarily reactive species.
- Radicals react easy with other radicals.

Reactive species - Definition

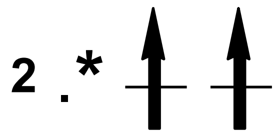
- Reactive species have a higher energy as the ground state.
- Highly reactive in chemical or biochemical reactions.
- Activation is necessary for a reaction under mild conditions.
- Example: Oxygen is reduced in the electron transport chain (mitochondria) via different intermediates to water.
- Reactive species are radicals and non radicals.

Oxygen - Groundstate

2 unpaired electrons,
biradical, triplet state



$^3O_2$ or •O_2•



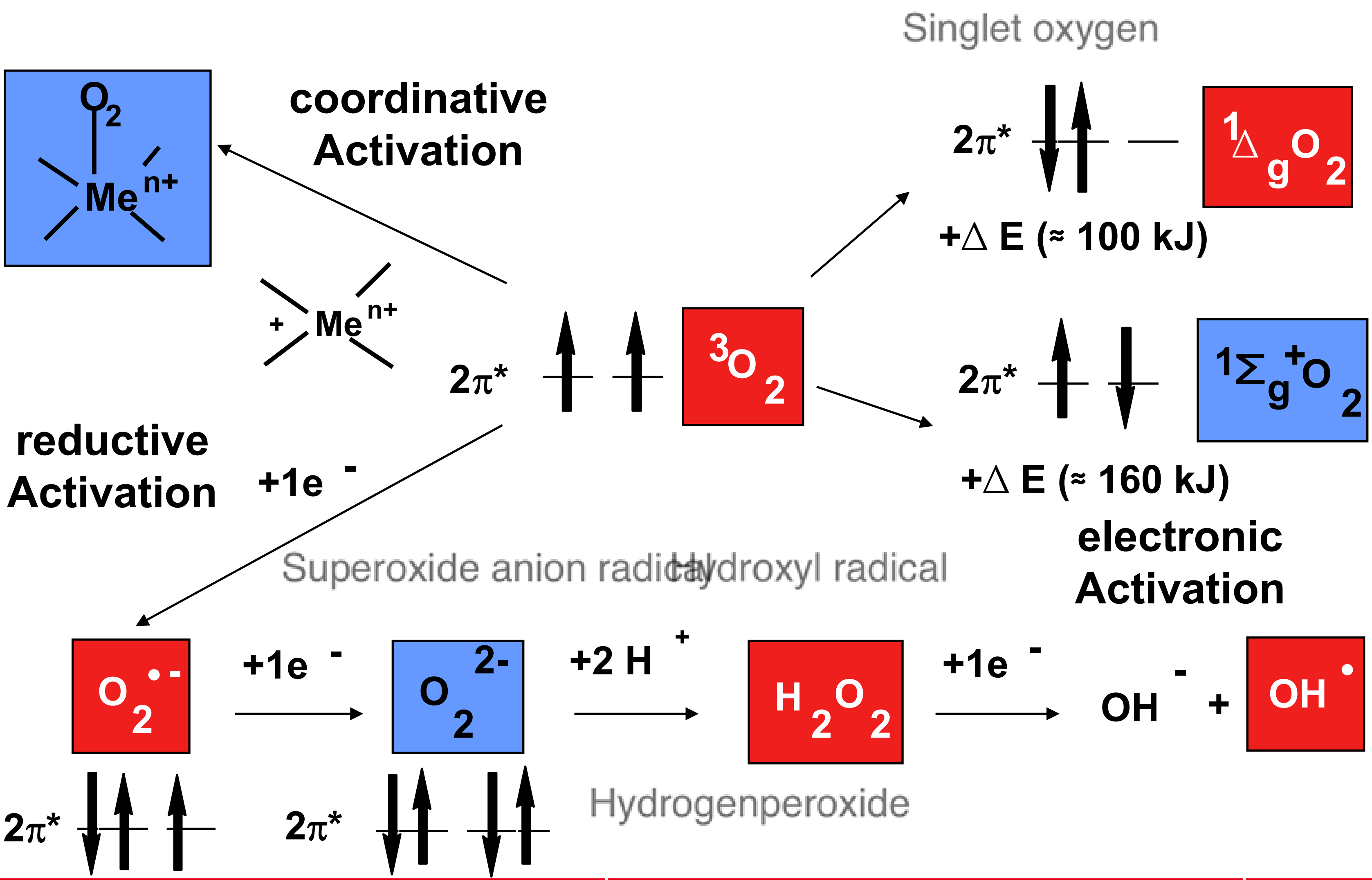
- Oxygen is unique in such a ground state
- Most of the other molecules have paired electrons

→ Spinbarrier between oxygen and the other molecules

→ No reactions, although thermodynamic possible

→ O_2 must be activated, the spinbarrier has to be removed

Activation of oxygen



Characterisation of superoxide

$O_2^{\bullet -}$

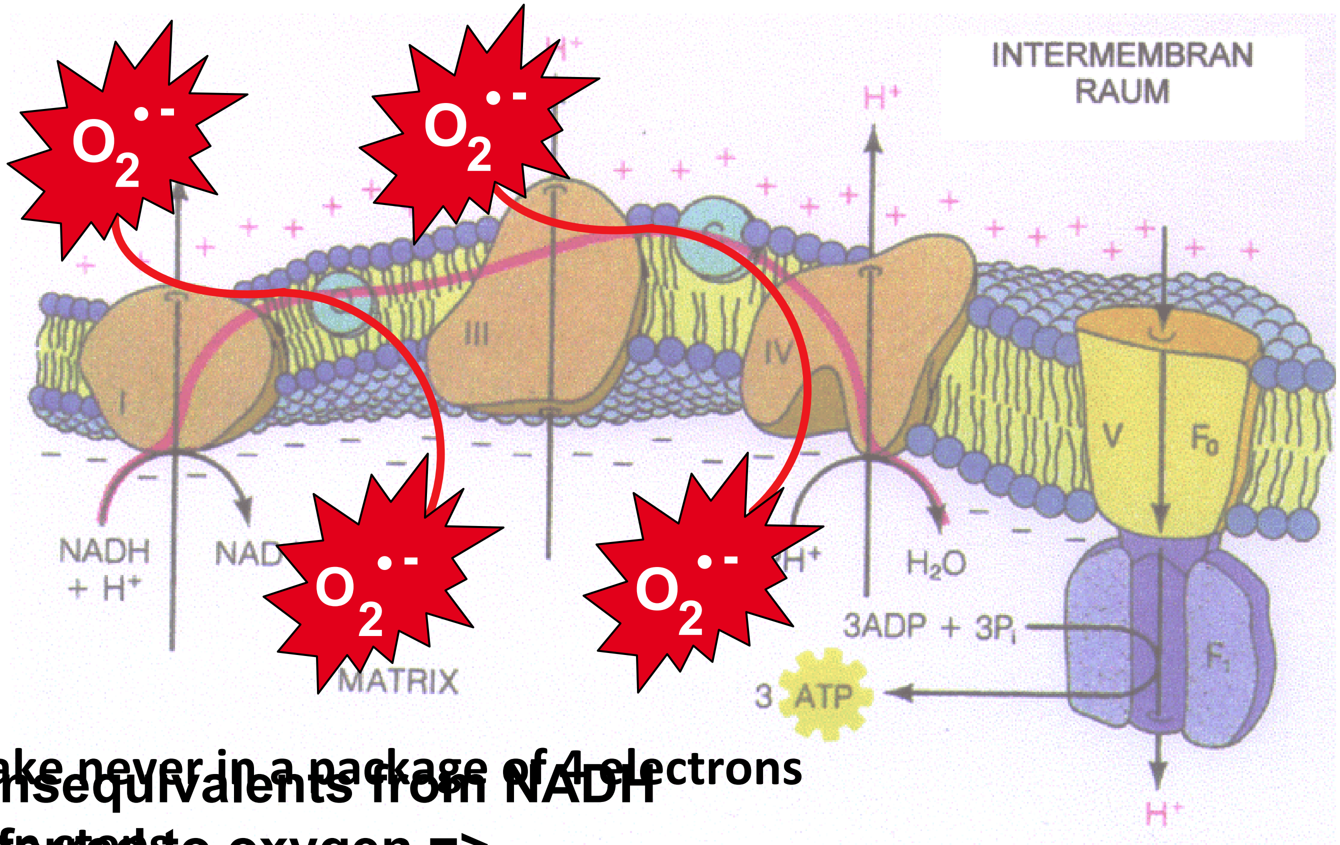
- Superoxide radical anion
  named by Pauling (1979)
  does not mean superoxidizing, but refers to the unusual configuration of the electrons.
- The anion is at physiological pH a very weak oxidizing agent (pK_a = 4,8).

$HO_2^{\bullet}$

- The protonated form is much more reactive, but this is not formed in the body.

➡ **The toxicity of superoxide is due to the following reactions of this species.**

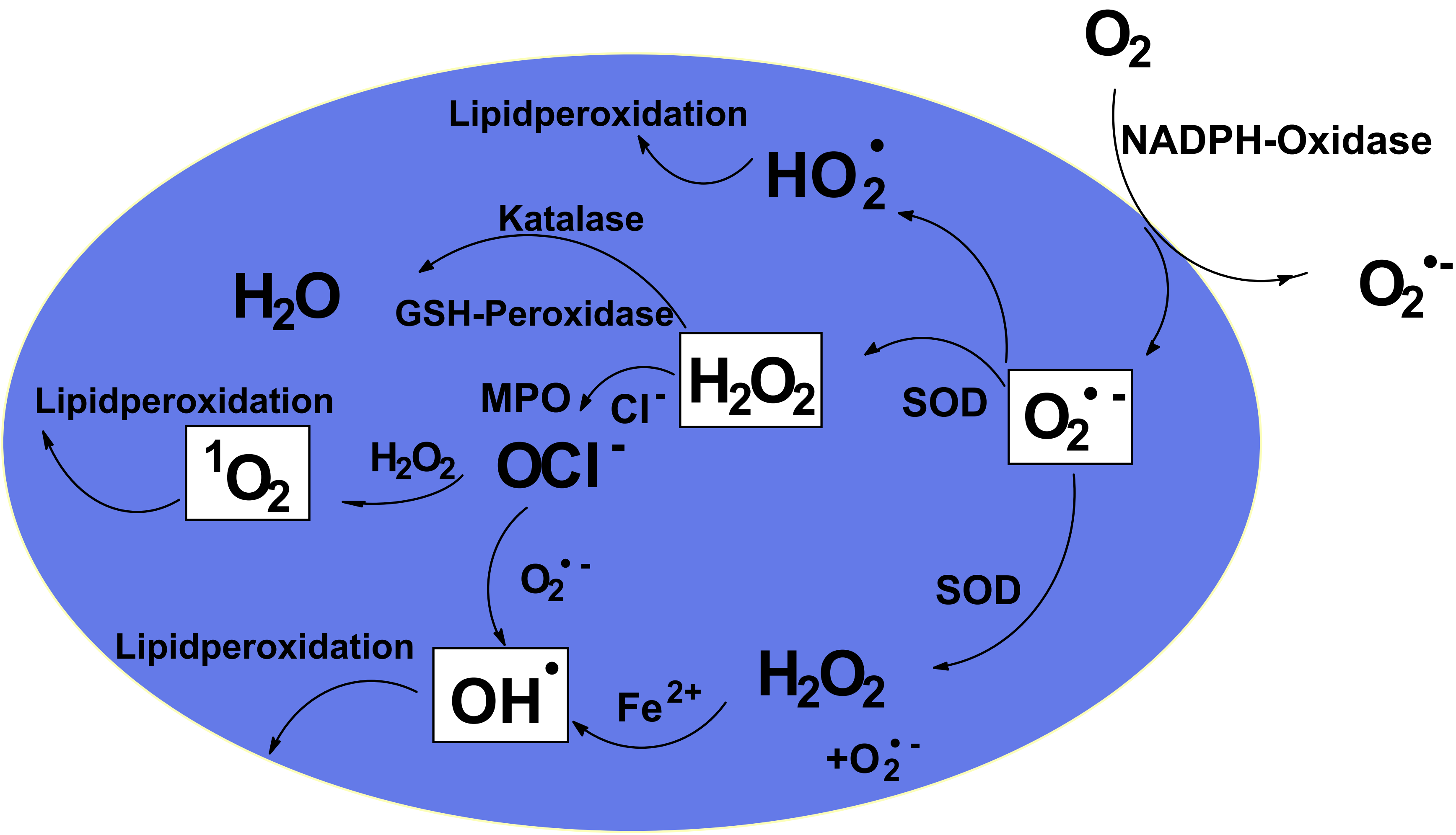
Mitochondrial electron transport chain



- **Electron uptake never in a package of 4 electrons**
- **4 One electron steps**
- **1. Step: Formation of the superoxide anion radical**

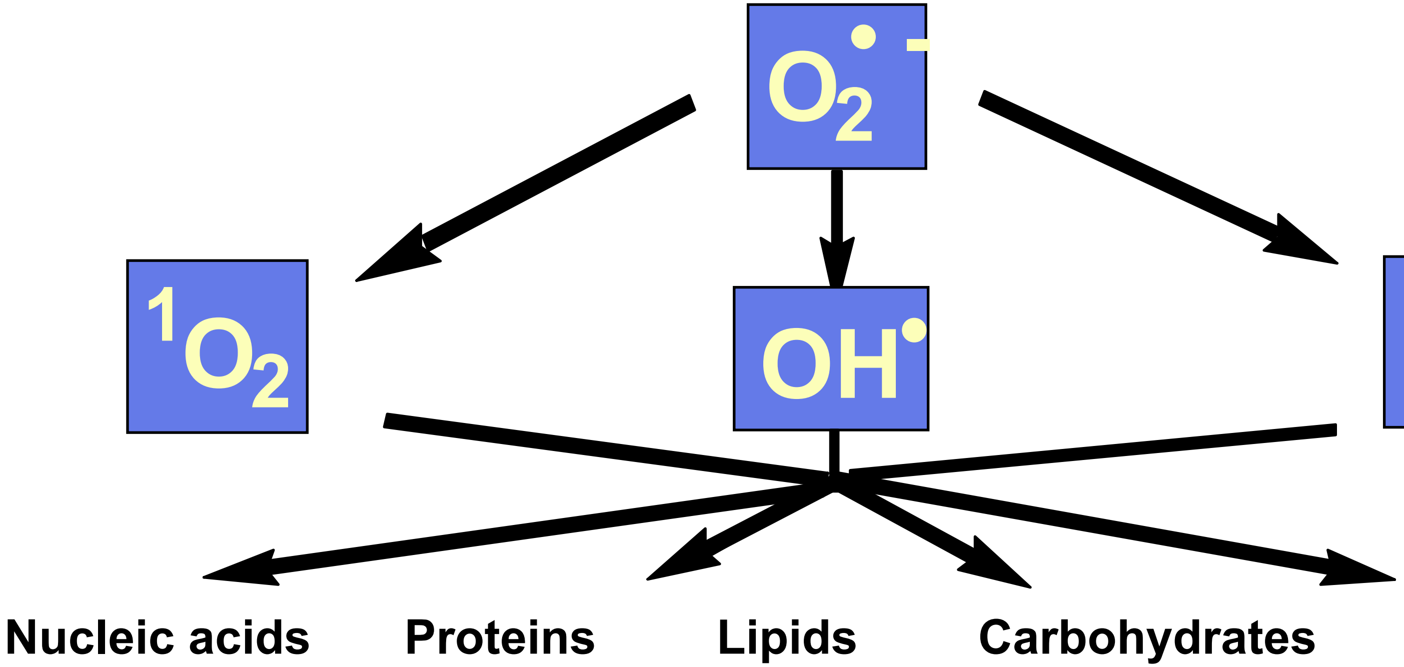
Reductionsequivalents from NADH are transferred to oxygen => Reduction to water
[4H] + O_2 --> 2 H_2O

Biochemical formation of reactive oxygen species



SOD = Superoxid-Dismutase MPO = Myeloperoxidase

Biological effects of reactive oxygen species



Cytotoxicity
Mutagenicity
Cancerogenesis
cytostatic effects

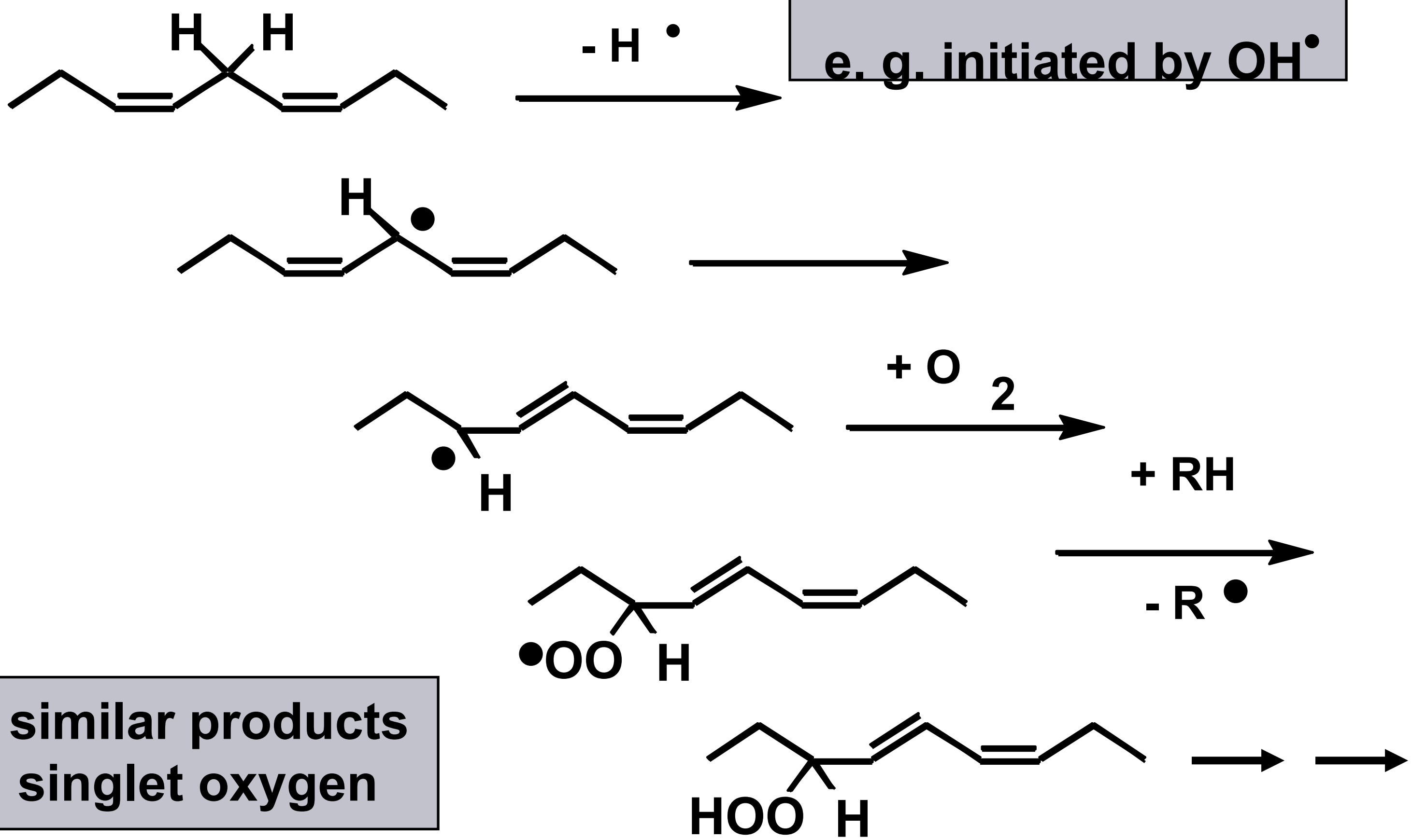
Enzymeinhibition
Enzymedestruction

Lipidperoxidation
Membrane damage
Arachidonic acid cascade

Desoxyribose damage
Hyaluronic acid degradation

Lipidperoxidation

e. g. initiated by OH•

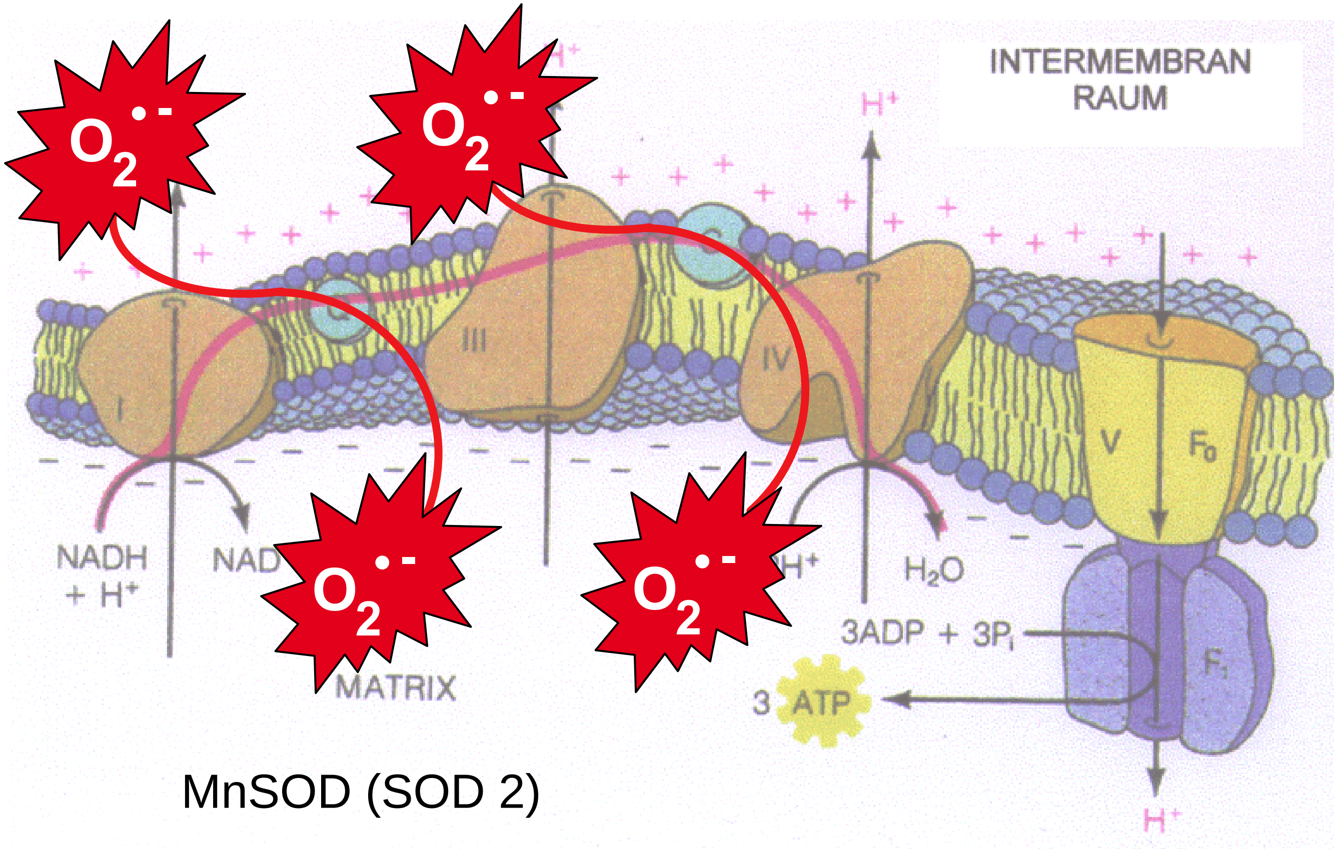


but, similar products with singlet oxygen

Formation of malondialdeyde

Enzymatic and non enzymatic antioxidants

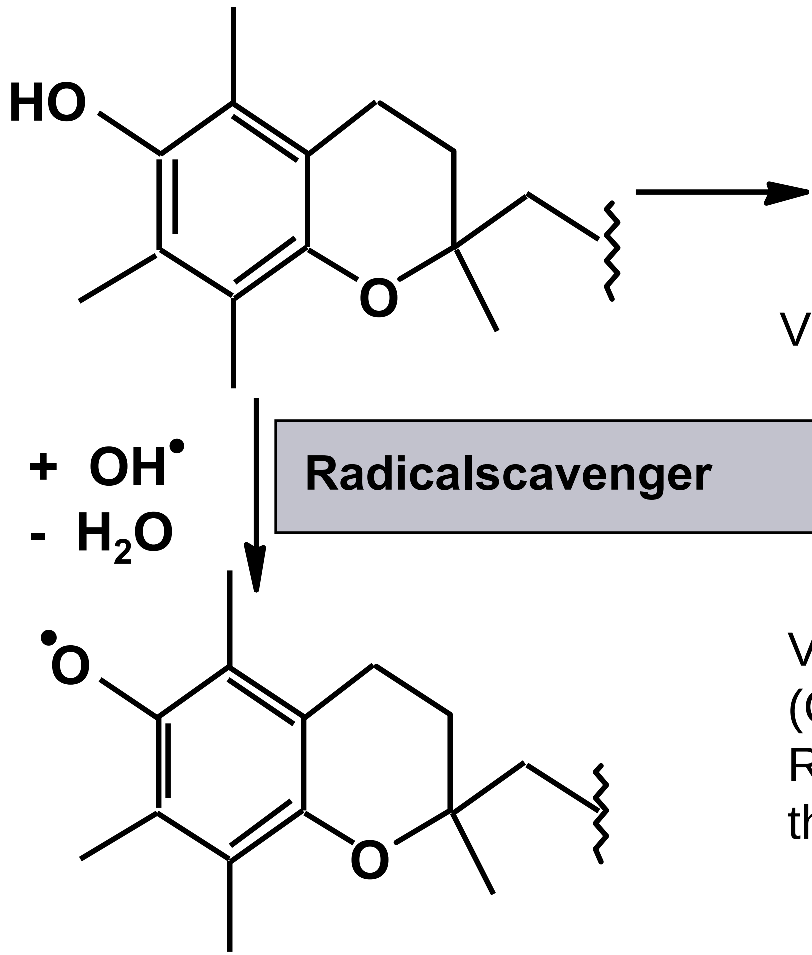
Enzymatic antioxidants



MnSOD (SOD 2)

- **SOD reaction** $O_2^{\bullet -} + O_2^{\bullet -} + 2\,H^+ \longrightarrow H_2O_2 + O_2$
- **Katalase reaction** $2\,H_2O_2 \longrightarrow 2\,H_2O + O_2$

Non enzymatic antioxidants
Vitamin E



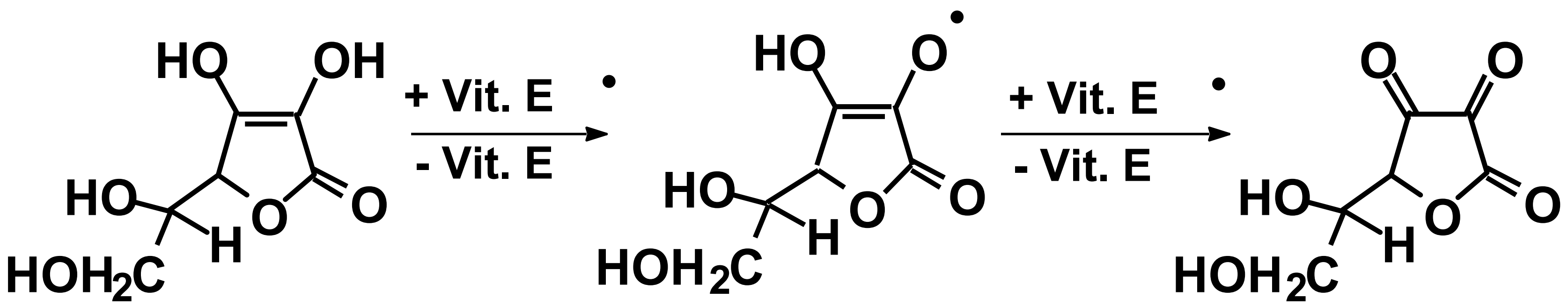
Chemical and physical quenching of $^1O_2$

Vitamin E (-Tocopherol)

Radicalscavenger

Vitamin E Radical
(Chromanoxyl-Radical)
Resonance stabilized,
therefore less reactive

Non enzymatic antioxidants
Vitamin E and Vitamin C



- Synergism with ascorbic acid
- Regeneration of Vitamin E
- Scavenger of active oxygen species

Superoxide toxicity and nitric oxide

- $NO^{\bullet}$ - another player in the game

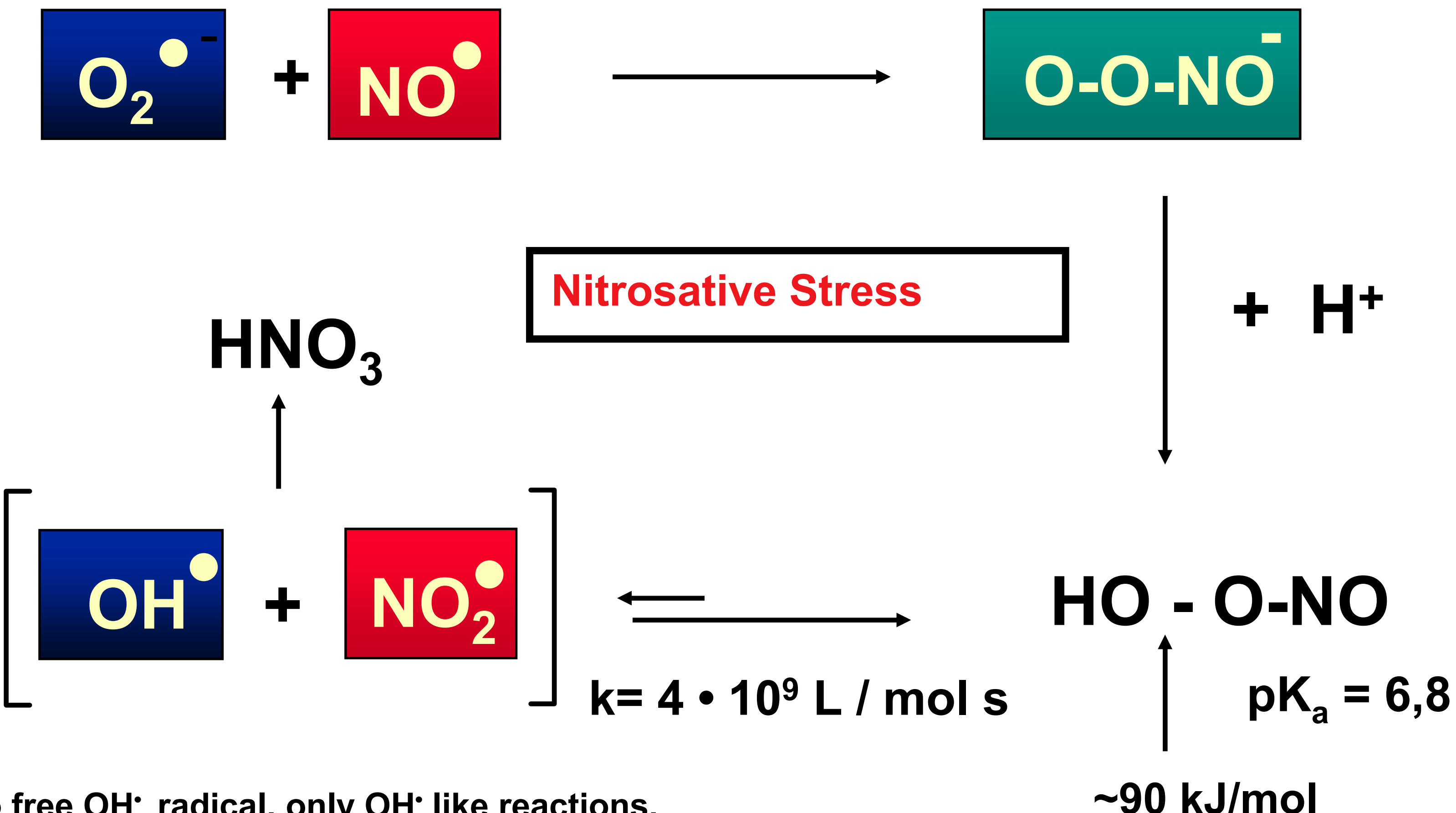
Reactive nitrogen species (RNS)

NO	Nitric oxide	Radical
NO_2	Nitrogen dioxide	Radical
$ONOO^-$	Peroxynitrite	no Radical

Biological interrelations of $O_2^{\bullet -}$ and $NO^{\bullet}$

- The reaction between $NO^{\bullet}$ und $O_2^{\bullet -}$ is three times than the reaction of $O_2^{\bullet -}$ and superoxid dismutase (SOD).
- $NO^{\bullet}$ is the only target molecule for superoxide, which can compete in the rate of reaction with the SOD catalyzed dismutation.
- The influence of SOD to reduce the biological half life of $NO^{\bullet}$, is a very strong hint for the biological importance of this reaction.

Interaction von $NO^{\bullet}$ und $O_2^{\bullet -}$ - Formation of peroxynitrite



No free OH$^{\bullet}$ radical, only OH$^{\bullet}$ like reactions,
Radicalscavenger have no influence on the reactions of peroxynitrite.

Biological targets of peroxynitrite

- Amino acids (tyrosine), lipids and nucleic acids.
- Cytotoxic weapon in macrophages.
- Peroxidation of membranelipids.
- Reaction with guanosine with strand brakes of the DNA.

Detection of radicals and reactive species

- Radicals
  - Hydroxyl radical, Superoxide, Nitric oxide
  - Direct: ESR-Spectroscopy
  - Spin trap methods (Generation of longlived radicals, e. g. Nitroxide radicals)
  - Other methods (Chemiluminescence enhanced with luminol, problem specificity)
- Reactive species
  - Hydrogenperoxide, Singlet oxygen
  - Peroxynitrite

ESR-Spectroscopy

Direct (Neuland and Pristner)

- Identification of an ESR signal after an achilles tendon lesion, that was intensified after shockwave application.
- This shows direct evidence, that the radical production is enhanced after shockwave application, but the intensity of the signal was too low for the exact identification of the nature of the radical.

ESR-Spectroskopy – Spin-Trap method

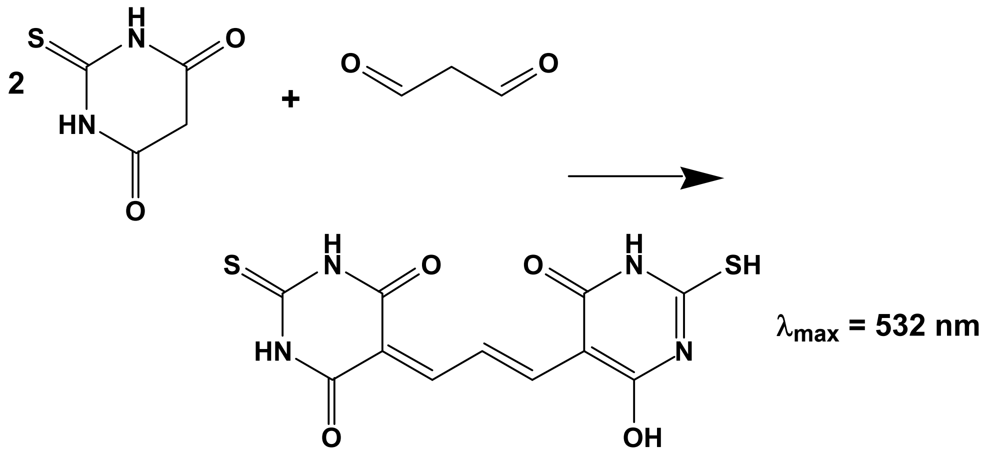
Indirect

- Bruno Fink and his company (NOXYGEN) had shown, that you can differentiate between radical species using different spin traps with a Pyrrolidine-skeleton (DMPOs).
- An unstable radical or even a reactive species like hydrogenperoxide will transferred into a more stable radical and could be detected in blood and *in vivo* too.
- This is a fascinating story, we try to find a way for cooperation.

„Fingerprint"-Methods

DNA-Alterations

- Alterations of the guanine in the DNA
  - **8-Hydroxy(desoxy)guanosine**
- Lipidperoxidation
  - Light emission (excited C=O-compounds)
  - Thiobarbituric acid assay (TBS), reaction of malondialdehyde mit TBS, problem: TBS is formed during the assay



Fingerprint“- Methods

- Isoprostanes - F_2-Isoprostane (Massspectroscopic measurement of the non physiological oxidationproducts of arachidonic acid; Problem: expensive devices)
- Aldehydes as products
  (e. g. 4-Hydroxy-nonenale)

Proteinoxidation

- Carbonyl-Assay
  (common assay with 2,4-Dinitrophenyl-hydrazine, not very specific)
- Nitration - 3-Nitrotyrosine

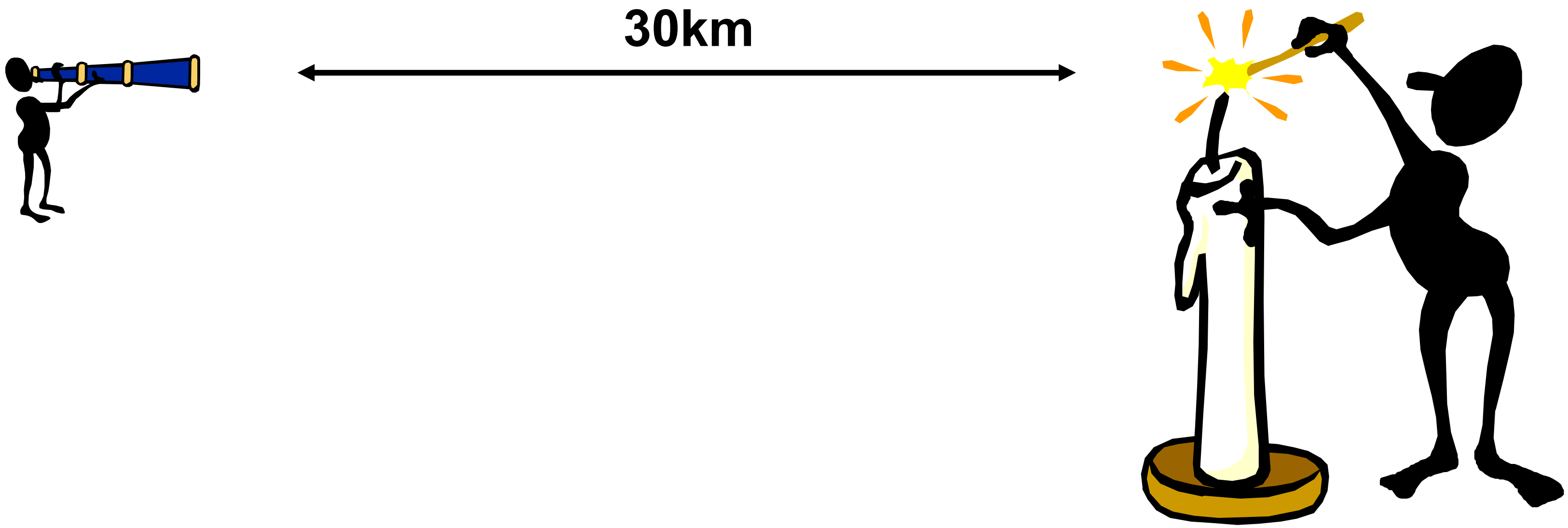
Detection of oxidative stress

Another physicochemical method

- Introducing a very sensitive method
  for ex vivo detection directly on the skin:
  Ultraweak chemiluminescence
  or ultraweak photonemission (UPE)

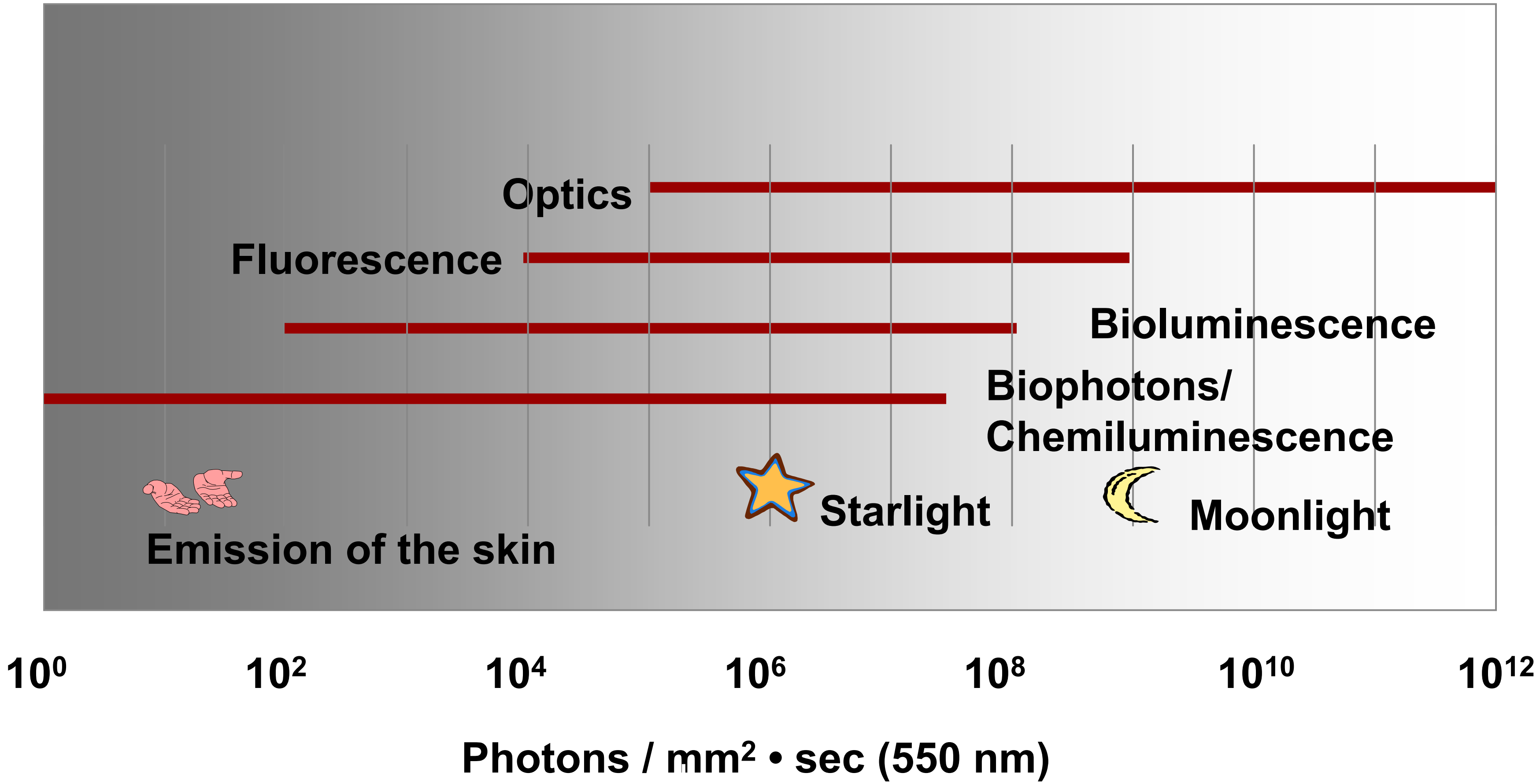
UPE-Measurement *in vivo*

The human skin emits light continously.



- This light is enhanced through external stress .

UPE –Measurement in comparison



Ultraweak Photonemission (UPE)

1. Spontaneous photonemission

2. Induced photonemission by external stress

External stress

Light (UV or visible)

Heat (e. g. sauna)

Cigarette smoke

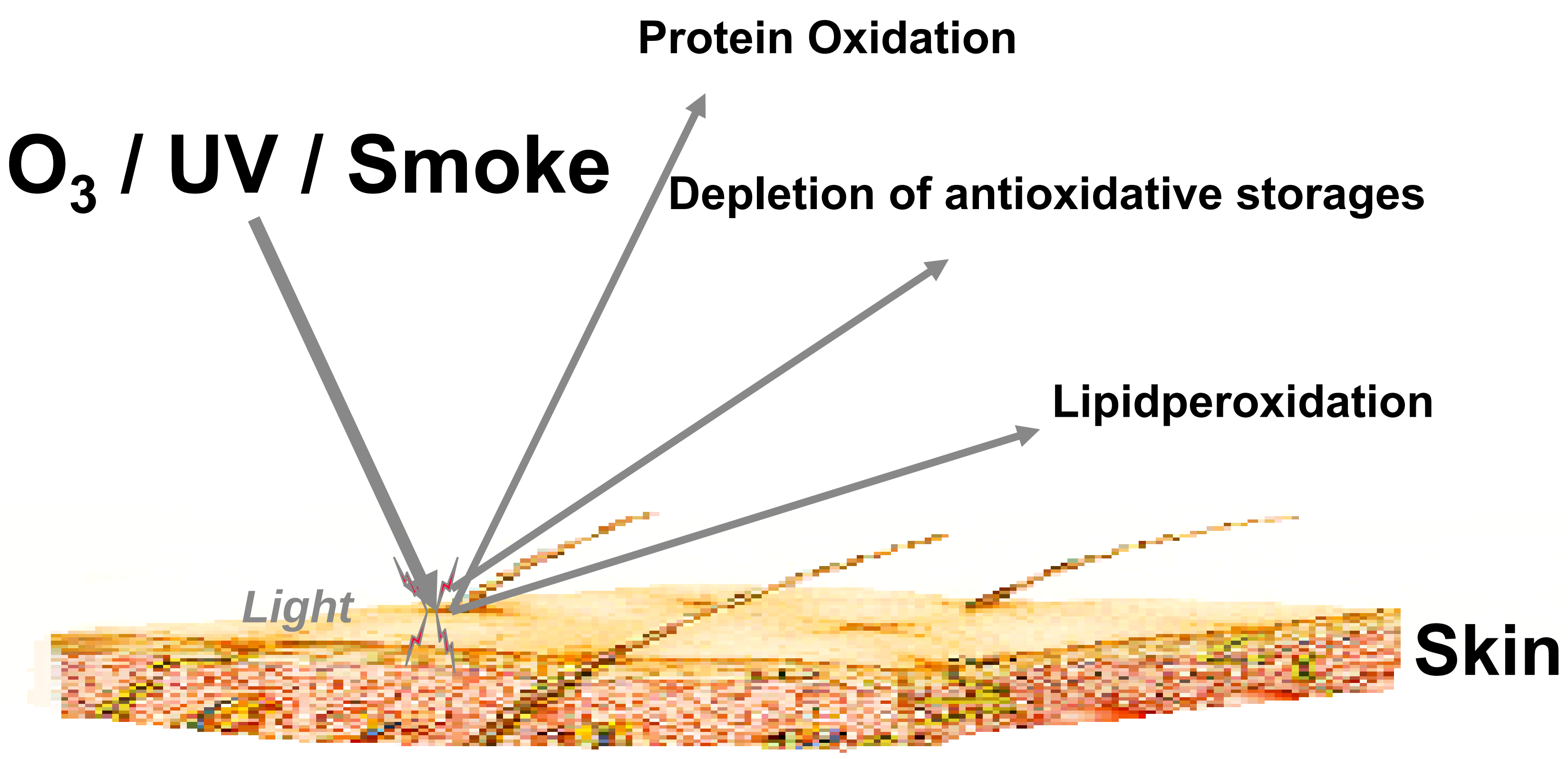
Ozone

Shaving

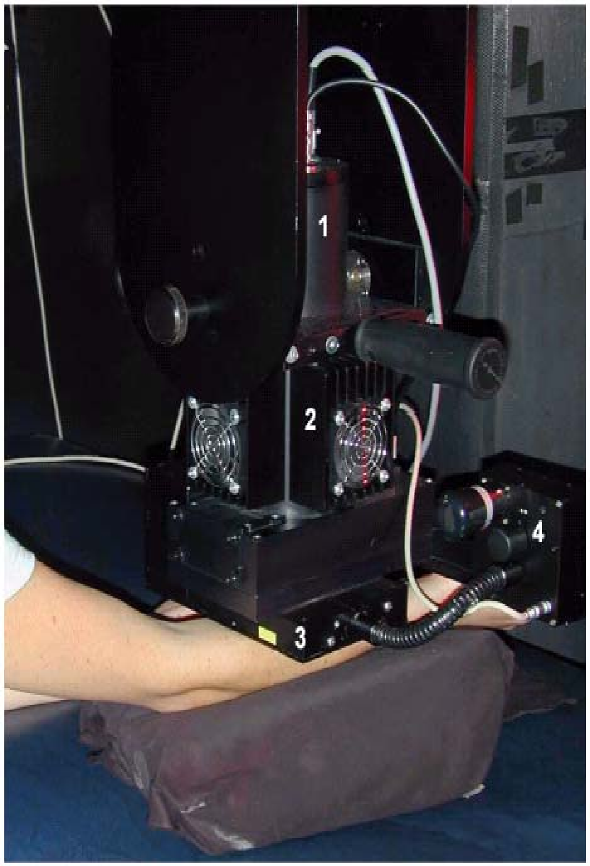
Shockwave application

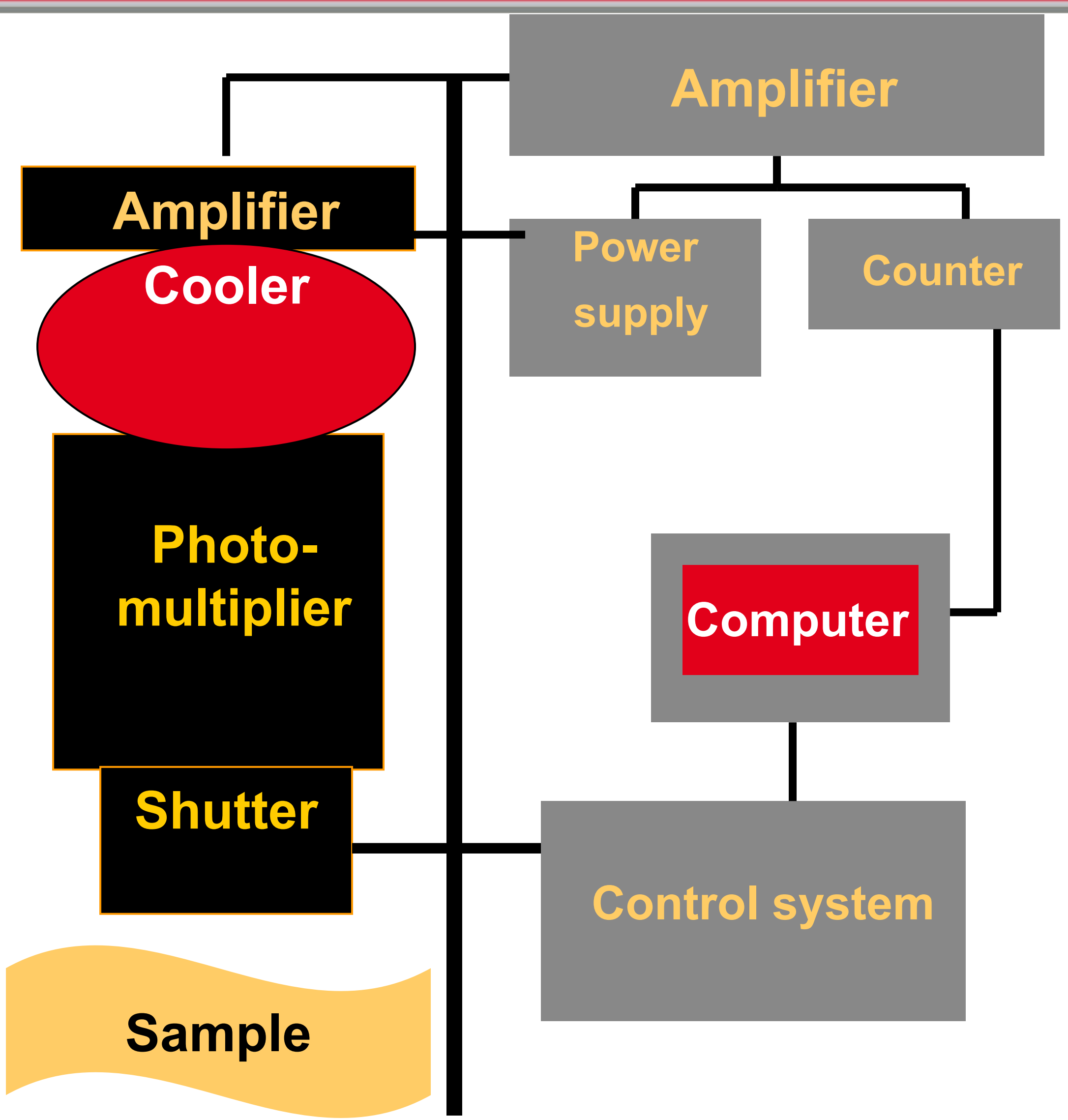
Aging

UPE - Model

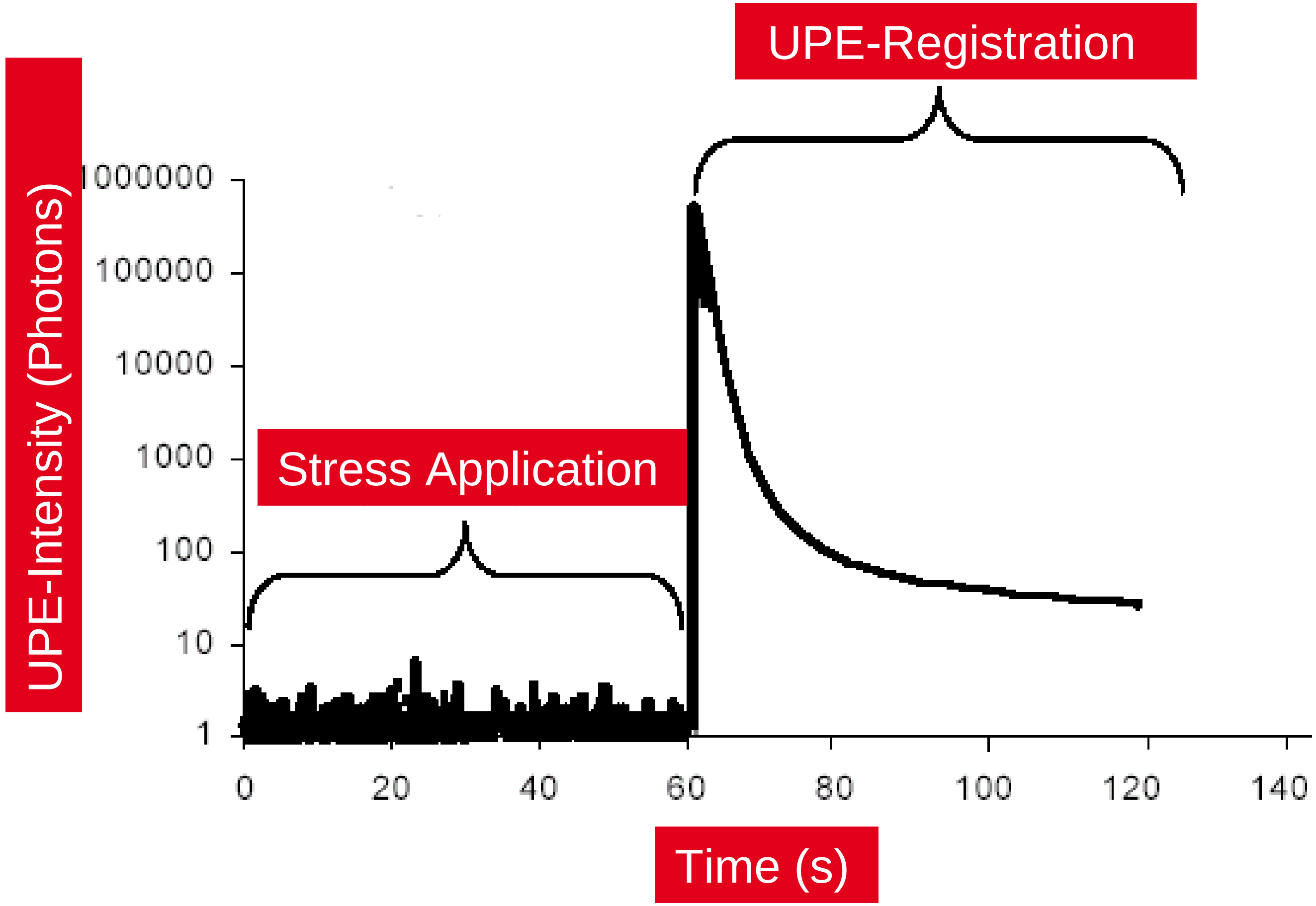

Protein Oxidation
O_3 / UV / Smoke
Depletion of antioxidative storages
Lipidperoxidation
Light
Skin

UPE measurement *in vivo*





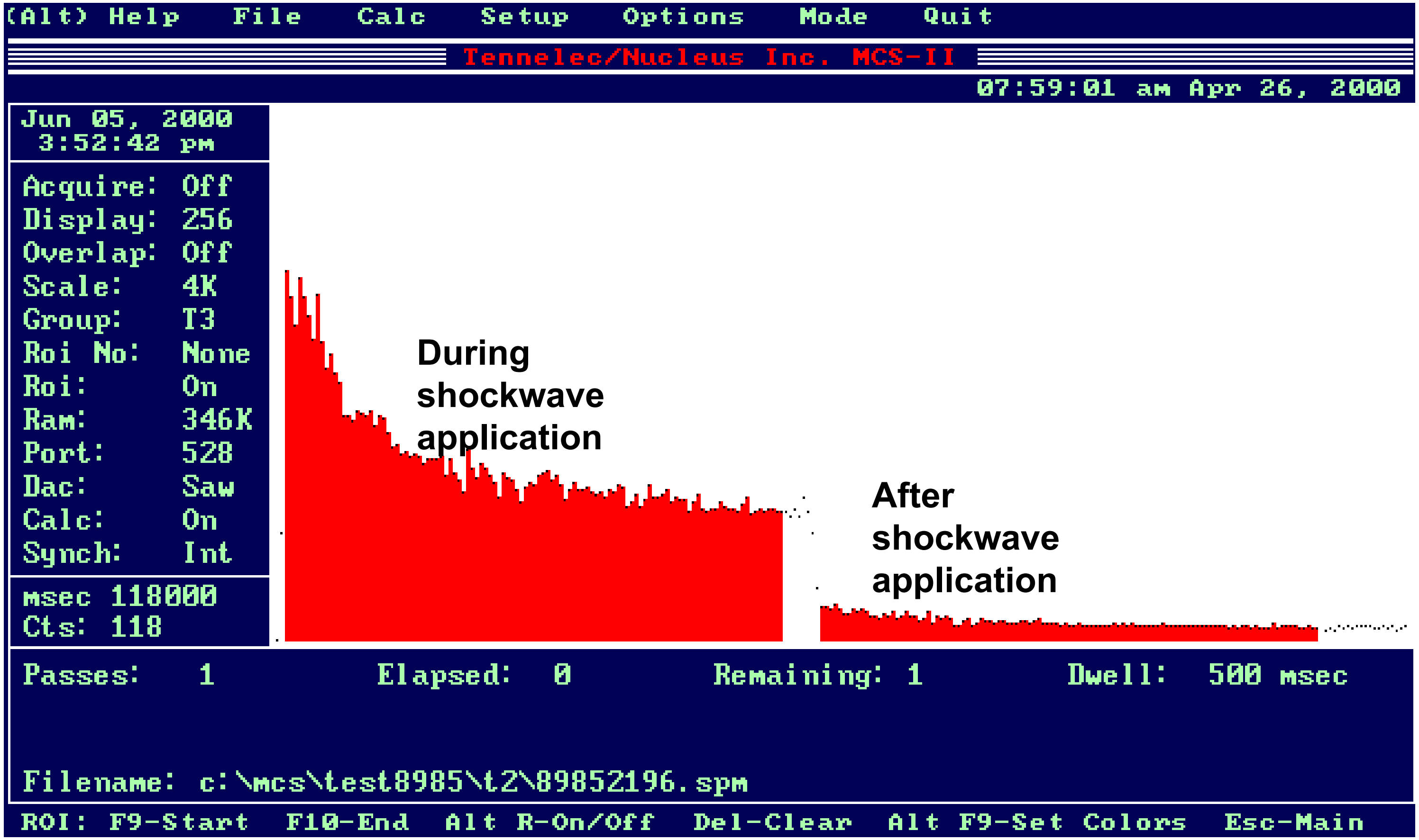
A typical UPE experiment



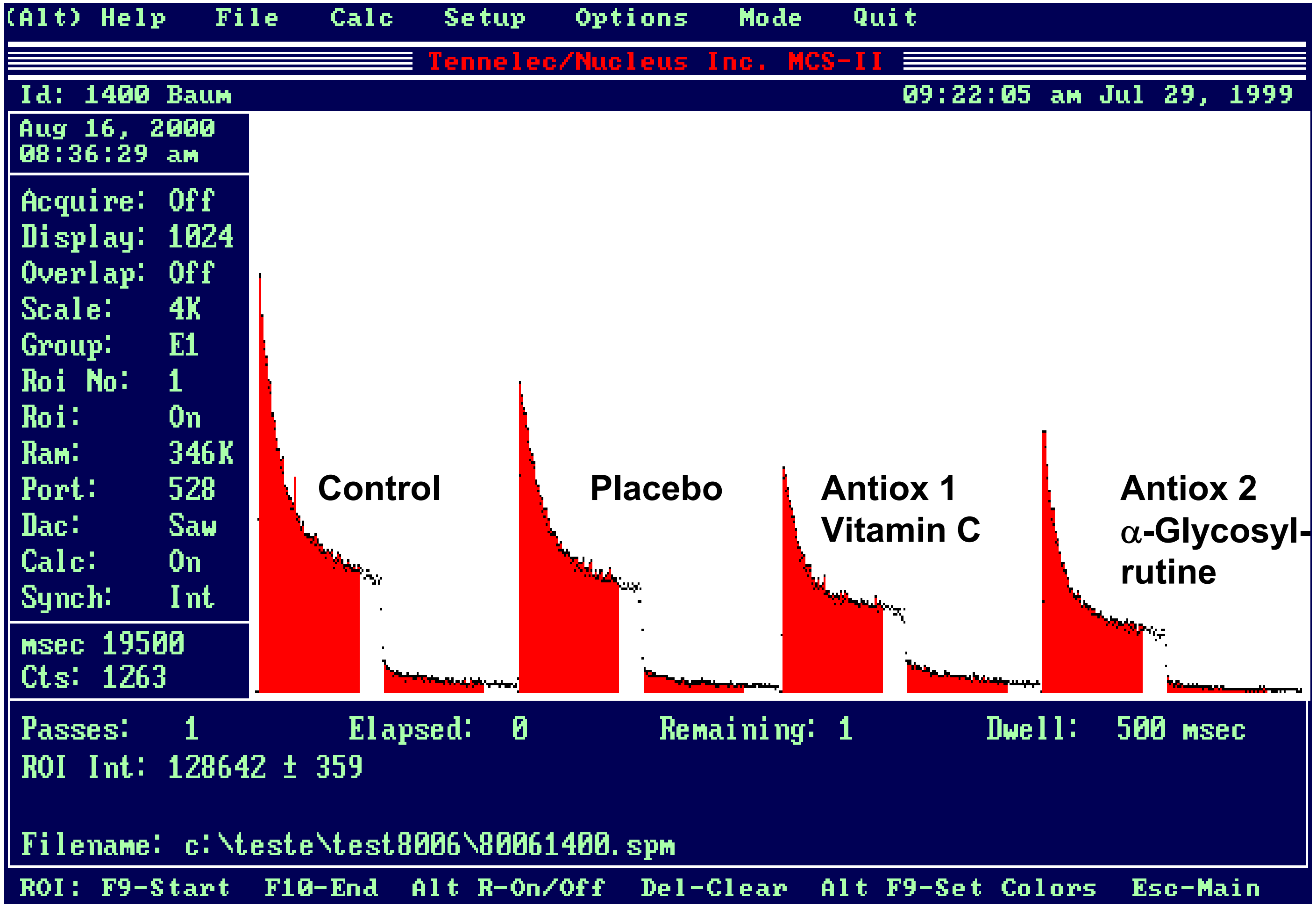
Circumstances to produce ROS and RNS

- Aging
- Involvement in more than 60 diseases
- External stress

Shockwave induced UPE *in vivo*



Nature of the UPE proofed with antioxidants



Measurement of reactive nitrogen species (RNS)

Nitric Oxide

- Chemical methods (Griess reaction)
- Diaminofluorescein (DAF-2) fluorescence
- Physicochemical methods
  - Electrochemical
  - ESR-spectroscopy
  - Chemiluminescense

Nitrogen dioxide

Peroxynitrite

Chemiluminescense of NO

Easy chemistry

- Oxidation of NO by ozone

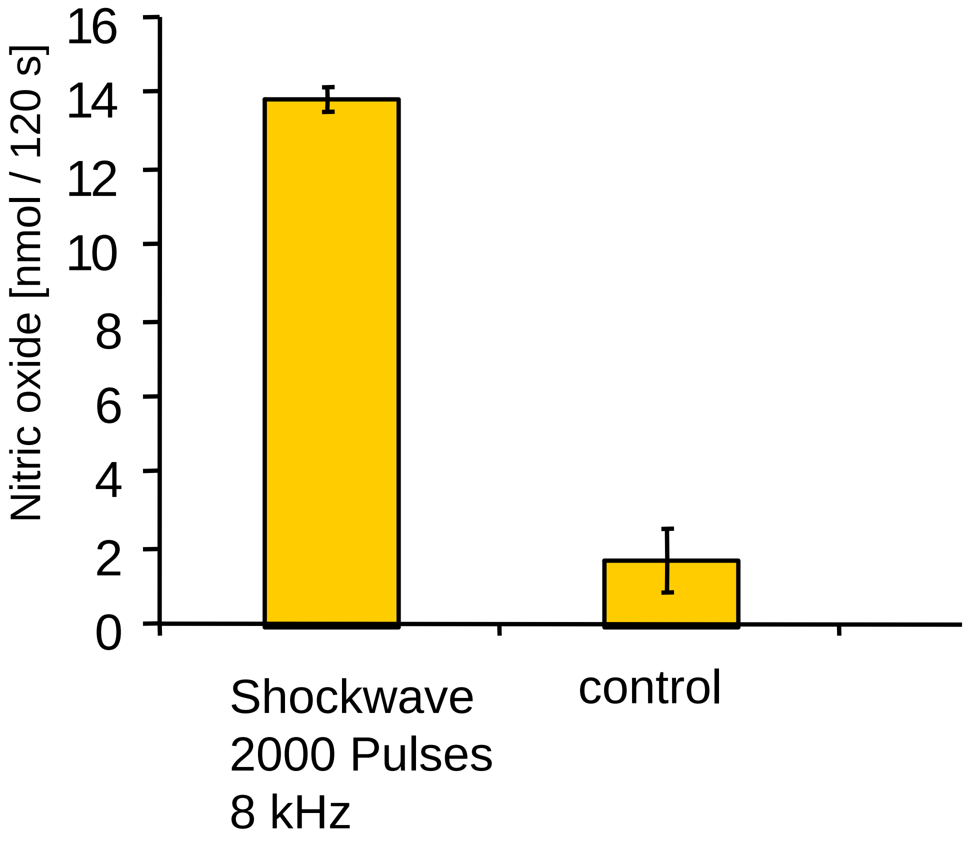
$$NO + O_3 \longrightarrow NO_2^* + O_2$$

- Activated nitrogen dioxide is desactivated under chemiluminescense, the intensity of this light is direct proportional to the amount of NO.

$$NO_2^* \longrightarrow NO + h\nu$$

Chemiluminescense equipment

NO Production after shockwave application



Mechanotransduction and shockwaves

Three major effects are observed after shockwave application

- Thermic measurable but low
- Chemical inducing chemical reactions like radical production
- Mechanical response to mechanoreceptors

What is Mechanotransduction?

Mechanotransduction is a mechanism by which cells convert mechanical stimuli into chemical activity via mechanoreceptors and following signaltransduction.

Our mechanical stimuli are shockwaves.

Our positive outcome is cell repair.

Mechanotransduction and Cell Repair. Still an unsolved Problem

Mechanotransduction

Shockwave Application ⬇ Mechanism?

Cell Repair

Our open question:
The biochemical pathway including the signaltransduction and the participation of reactive species

Possible Rationale

Shockwave Application → Tissue

Mechanotransduction

Oxidative signaling measured by induced UPE

Cell repair

Angiogenesis

Measuring of nitric oxide

Further insights

We looked in more detail in the biochemical pathways involving oxidative signaling

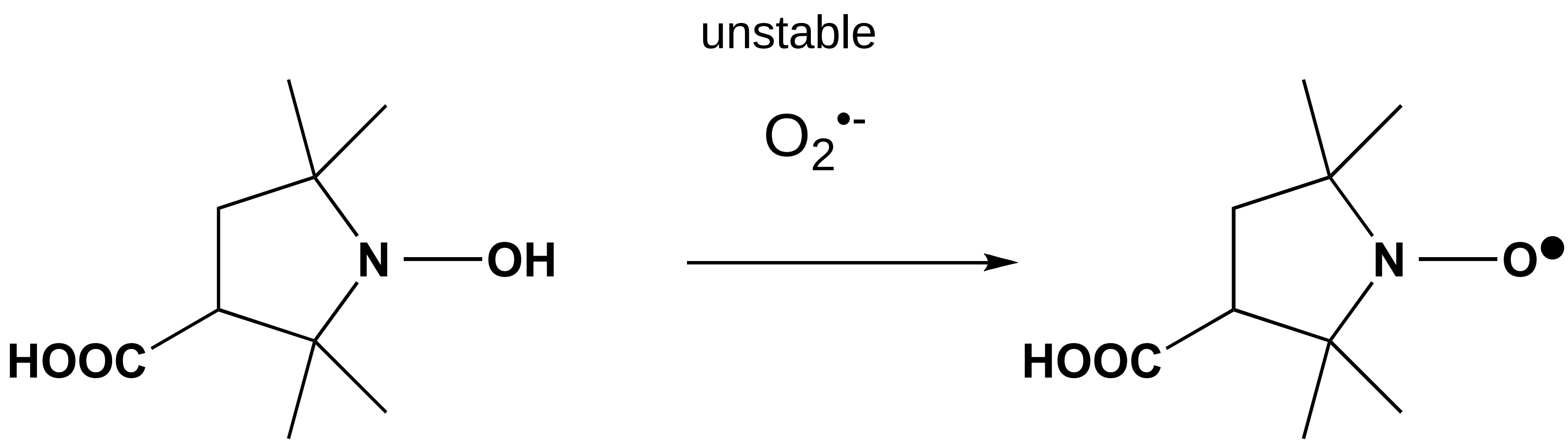
Mechanotransduction

Heat shock proteins like HSP 60 are upregulated

Where is the oxidative signaling involved?

Stimulation of the migration of mesenchymal stem cells

More sensitive detection methods: Specific spin-traps



Hydroxylamine derivative

Nitroxide radical
stable for hours

Thank you for your attention

Faryar Khabiri

Weng Mei

Rolf Hagens

Helmut Neuland

BDF ●●●●

Beiersdorf