LONGEVITY & PROTECTIVE BEAUTY

Glorydermal® **Guard**

Holistic Skin Protection
through Enzymes

Introduction

In daily life our skin is exposed to many different influences, that make the signs of time visible...

Two key influences are responsible for most of the **aging processes** and are closely related to each other:

Conclusion: Clinical signs of aging are essentially influenced by extrinsic factors, especially sun exposure. Indeed UV exposure seems to be responsible for 80% of visible facial aging signs.

Flament, F. *et al. Clinical, Cosmetic and Investigational Dermatology* **6**, 221–232 (2013).

UV radiation and **free radicals**

are the major causes of premature skin aging due to e.g. **DNA damage** and **oxidation processes** which lead to aging signs like photo aging, wrinkles, …

Introduction

That is why our skin needs a reliable
repair and protection system

UV radiation/DNA damage

Free radicals/oxidation processes

Active Description – Enzymes and Enzyme-like Actives

UV radiation and **free radicals** are both very well-known natural influences of **ancient origin**.

Since the beginning of life, organisms are depended on using effective **repair and protective mechanisms** to counteract these influences to survive.

Enzymes play an essential role in these processes. They **enhance and accelerate** many biochemical reactions and they have **important functions in the metabolism of organisms**.

They are **not used up** over many reaction cycles and therefore they offer a **long-term powerful efficacy**.

Enzymes could be called the "conductors of life".

Active Description – Enzymes and Longevity

Enzymes could be called the “conductors of life”.

Enzymes are directly linked to longevity!

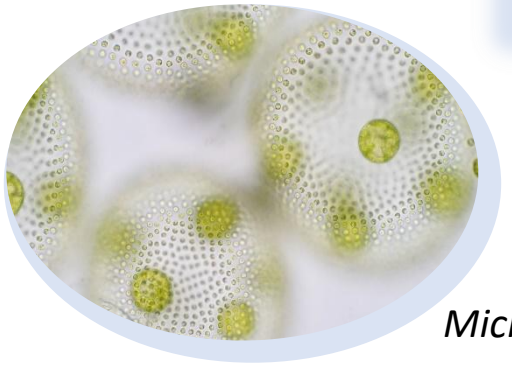
Enzymes are key regulating elements in the body. Active ingredients with **enzymes** or **enzymatic activity** thus **contribute to longevity at a crucial point**.

As **DNA repair** and **protection against oxidative processes** play a significant role in **preventing premature skin ageing**, the enzymatic components of Glorydermal® Guard **ideally match the concept of longevity**.

Active Description – Learning from Nature: Two Enzymatic Partners

DNA repair

Repair enzyme photolyase



Microalgae extract

Antioxidant enzyme

Iron peptide

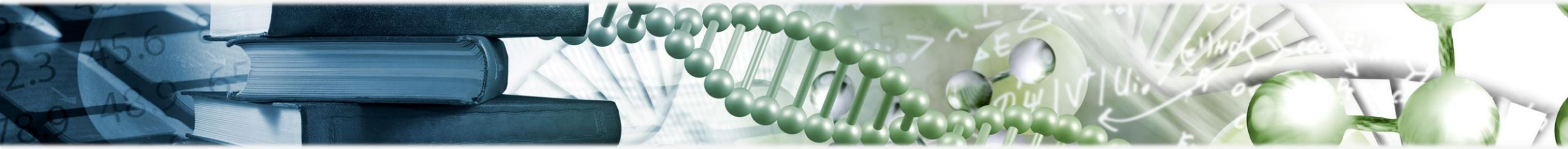
Neutralisation of free radicals

Synergistically and continuously acting complex consisting of **two enzymes**:

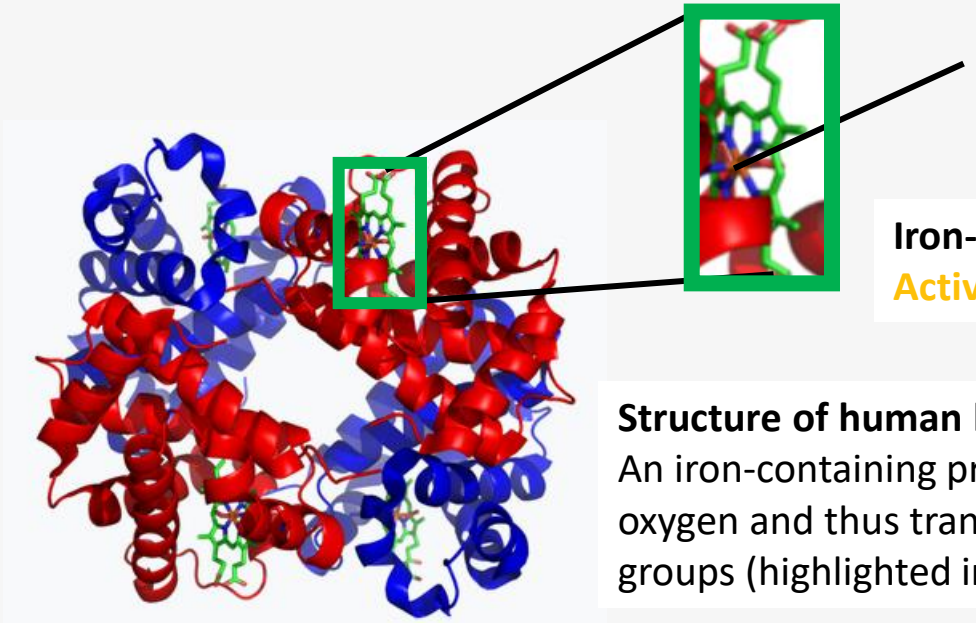
- the **repair enzyme photolyase** (microalgae extract) and
- an **antioxidant enzyme** (iron peptide)

encapsulated in liposomes for improved penetration

Active Description – Learning from Nature



The **iron peptide** in Glorydermal® Guard has a similar structure to the **active centres of hemoglobin**



Iron ion in the centre of the heme group

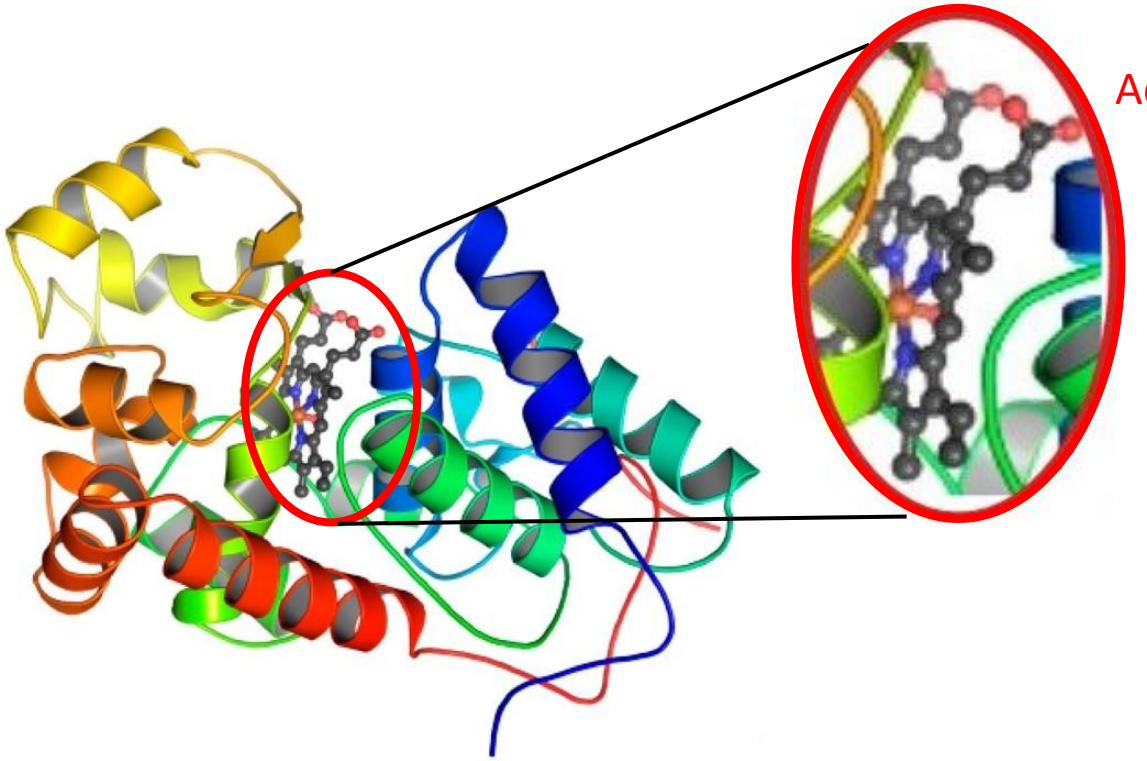
Iron-containing heme groups:
Active centres which bind oxygen via the **iron ion in the centre**.

Structure of human hemoglobin:
An iron-containing protein complex in the red blood cells of vertebrates, which reversibly binds oxygen and thus transports it in the bloodstream. An iron ion is centred in each of the 4 heme groups (highlighted in green).

Source: https://en.wikipedia.org/wiki/Hemoglobin

Active Description – Learning from Nature

The iron peptide in Glorydermal® Guard is
the first and, to date, only synthetic peroxidase-like active for use in cosmetics.



3D Structure of Horseradish Peroxidase
*(Danielson, A. et al. Investigating the Mechanism of Horseradish Peroxidase as a RAFT-Initiase. Polymers **10**, 741 (2018).)*

Active centre of the horseradish peroxidase with iron core

The iron peptide in Glorydermal® Guard **mimics the active centre** of the peroxidase.

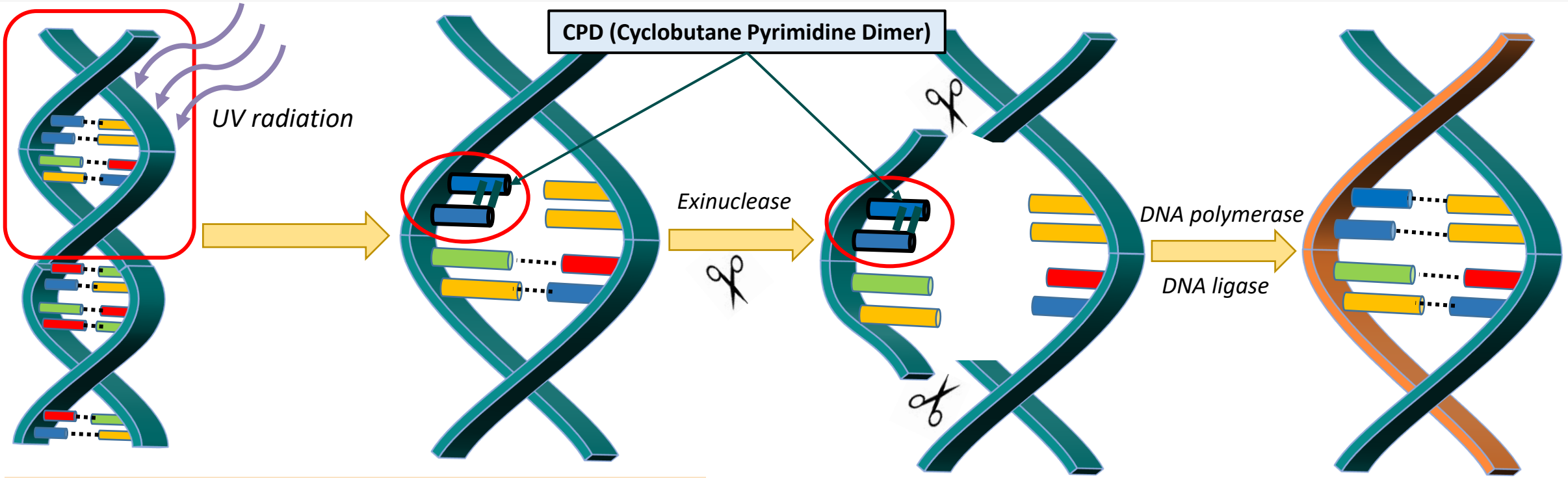
Focus on active core of the enzyme allows **better skin penetration properties** (size!) and **high efficacy**.

Synthetic pathway offers **highly standardised quality and quantity**, which is not possible by common enzyme extractions from natural sources.

Peroxidases are enzymes that catalyse the reduction of peroxides (usually hydrogen peroxide).

Active Description – Excursus DNA Repair

Human Repair Mechanism



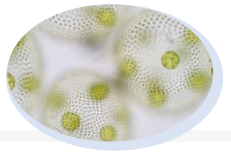
UV radiation can separate opposite base pairings in the DNA double helix. This results in reactive base ends that can erroneously combine adjacent to each other on the same helical strand. Due to this mechanism linkage of adjacent thymine bases to form a Cyclobutane Pyrimidine Dimer (CPD) is possible. CPDs are the most common DNA damage (DNA construction error) caused by UV radiation.

The enzyme exinuclease recognises this DNA construction error and generously separates the affected DNA strand around the faulty linkage.

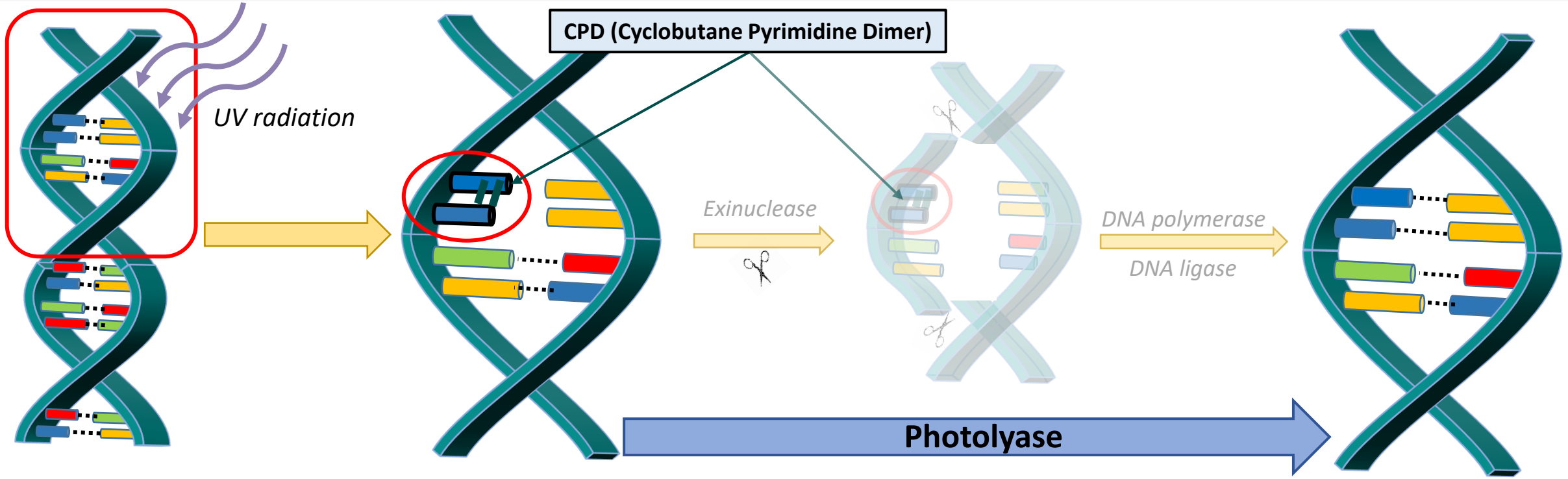
The complementary DNA strand is then re-synthesised by DNA polymerase and the ends of the previous cut edges are closed by DNA ligase. The DNA damage is thus repaired.

Mechanism according to nucleotide excision repair | Nobel Prize Chemistry 2015

Active Description – Repair Enzyme Photolyase

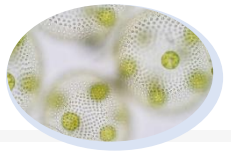


Human Repair Mechanism

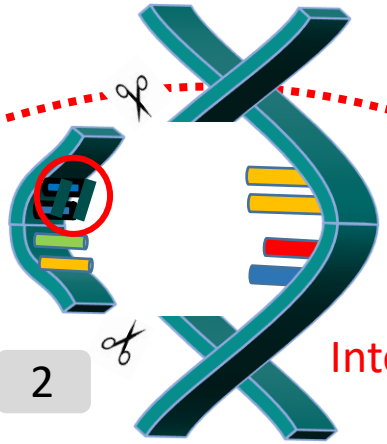


The repair enzyme photolyase acts **analogously to the human repair mechanisms**, but repairs the DNA **directly by cleaving the CPD bonds** without the need to remove the DNA strand. As a result, this repair is **much faster** than the human repair mechanisms, which can thus be supported very effectively. This is due to the evolution of microalgae, which developed an **effective protection and repair system against intense UV radiation billions of years ago**. Photolyase is particularly active in the range of **blue radiation (blue light)** and acts over **many repair cycles** (enzymatic action, is not used up in the process).

Active Description – Enzymes: Continuously Operating Accelerators

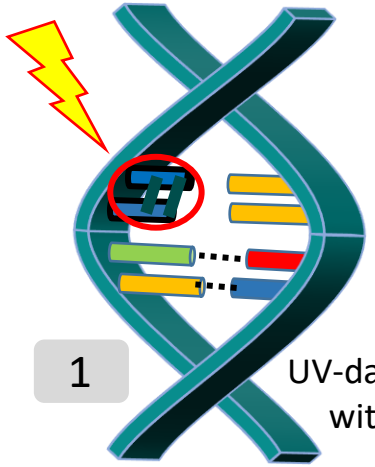


Slow diversion
without the repair enzyme photolyase



2 Intermediate

**than repair in untreated skin cells, (see diversion with intermediate (2))*

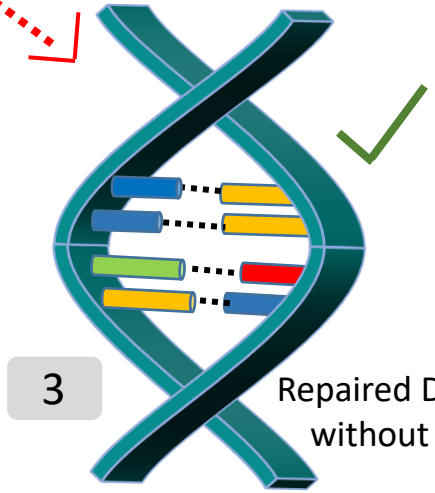


1 UV-damaged DNA with CPDs

Fast repair
with the repair enzyme photolyase

Direct repair without intermediate

10-100 times faster*
with repair enzyme photolyase



3 Repaired DNA without CPDs

Repair enzyme photolyase is not used up

Many repair cycles possible

Active Description – Antioxidant Enzyme

Antioxidant enzyme (iron peptide):

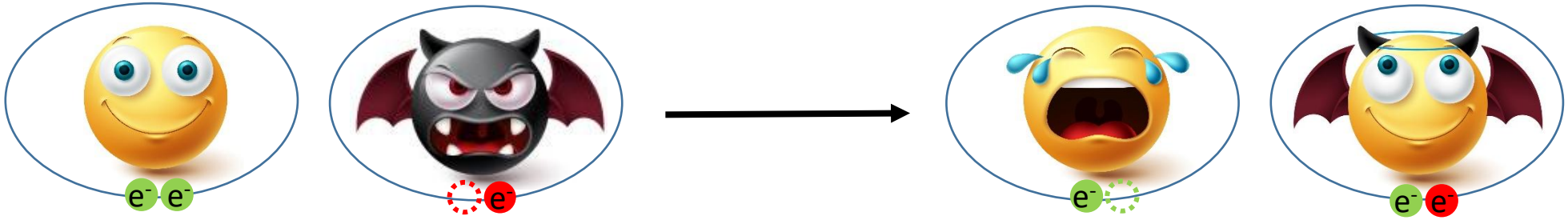
Reactive Oxygen Species (**ROS**), which include free radicals, are **continuously neutralised**.

This long-term antioxidant acts like an enzyme.
It is **not used up** and **regenerates itself**.

→ **Long-term radical protection**

Excursus Free Radicals – **Action**, Neutralisation, Long-term Radical Protection

Action of free radicals **without antioxidants**



Molecule with paired electrons

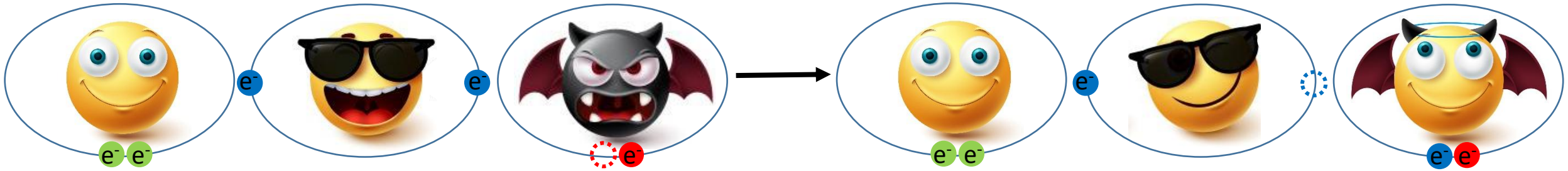
Free radical without paired electrons

Molecule without paired electrons

Free radical with paired electrons

Excursus Free Radicals – Action, **Neutralisation**, Long-term Radical Protection

Neutralisation of free radicals **with common antioxidants**



Molecule with paired electrons

Antioxidant with flexible electron to share

Free radical without paired electrons

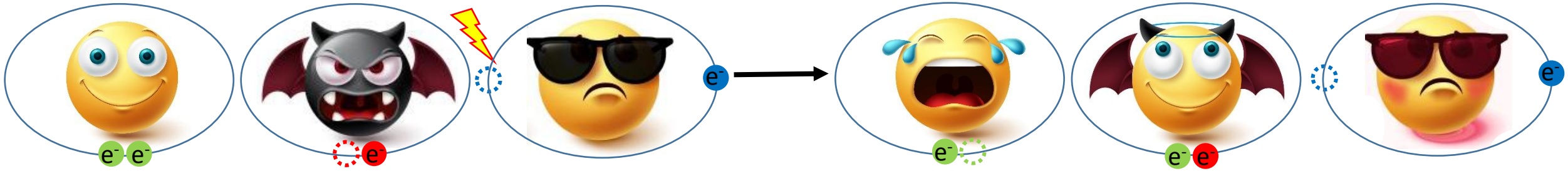
Molecule with paired electrons

Used up antioxidant without flexible electron to share

Neutralised free radical with paired electrons

Excursus Free Radicals – Action, Neutralisation, Long-term Radical Protection

Limits of neutralisation of free radicals **with common antioxidants**



Molecule with paired electrons

Further free radical without paired electrons

Used up antioxidant without flexible electron to share

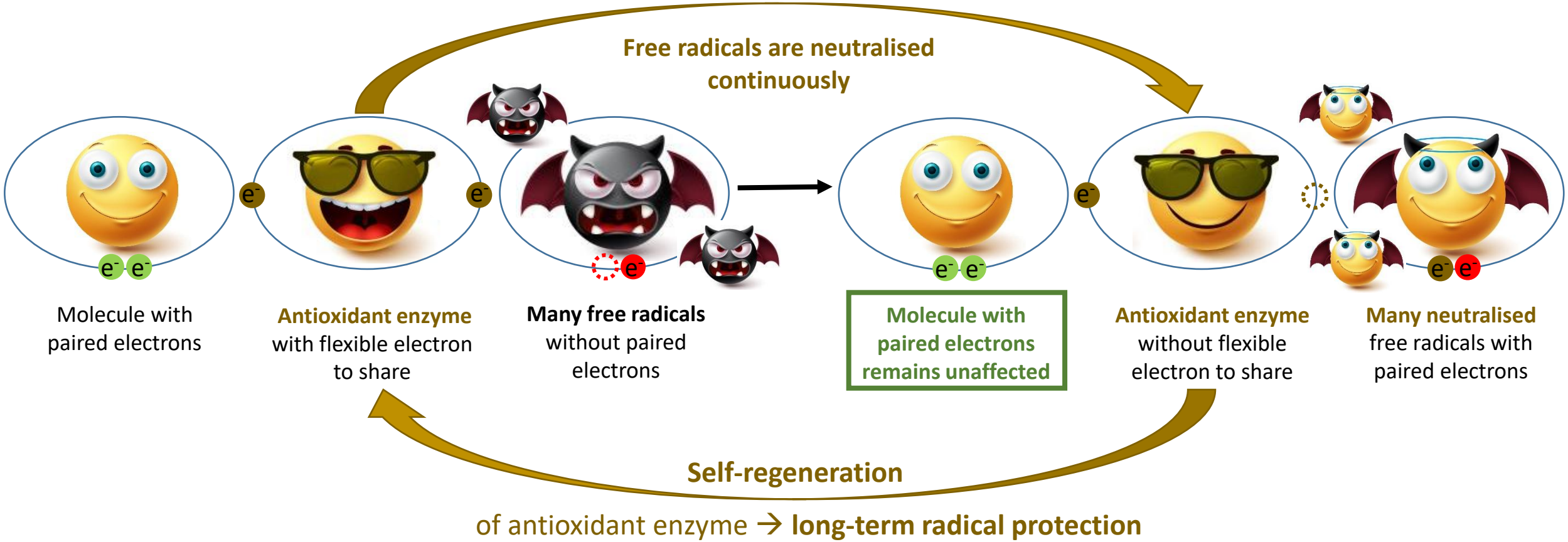
Molecule without paired electrons

Further free radical with paired electrons

Used up antioxidant without flexible electron to share – **no regeneration**

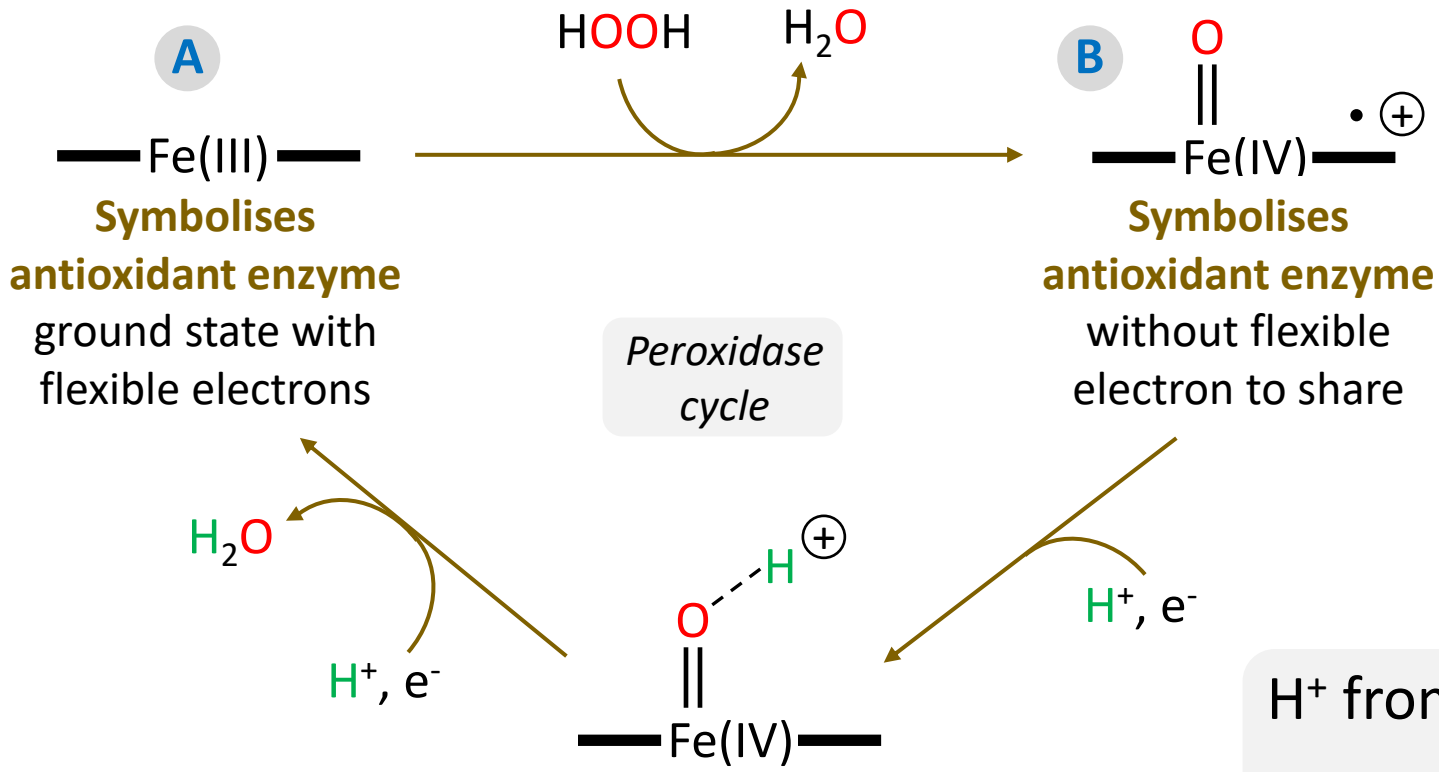
Excursus Free Radicals – Action, Neutralisation, **Long-term Radical Protection**

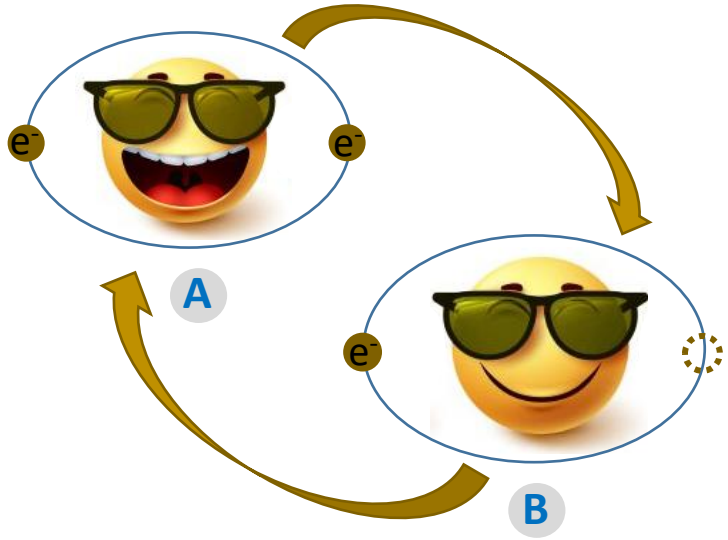
Neutralisation of free radicals **with antioxidant enzyme in Glorydermal® Guard**



of antioxidant enzyme → **long-term radical protection**

Excursus: Self-regeneration of the Antioxidant Enzyme





H^+ from autoprotolyse of water:

$$2\ H_2O \rightleftharpoons 2\ H^+ + 2\ OH^-$$

Adapted from the peroxidase cycle of horseradish peroxidase (Berglund, G., Carlsson, G., Smith, A. *et al.* The catalytic pathway of horseradish peroxidase at high resolution. *Nature* **417**, 463–468 (2002).)

Stability of the Oxidation State Fe(IV)

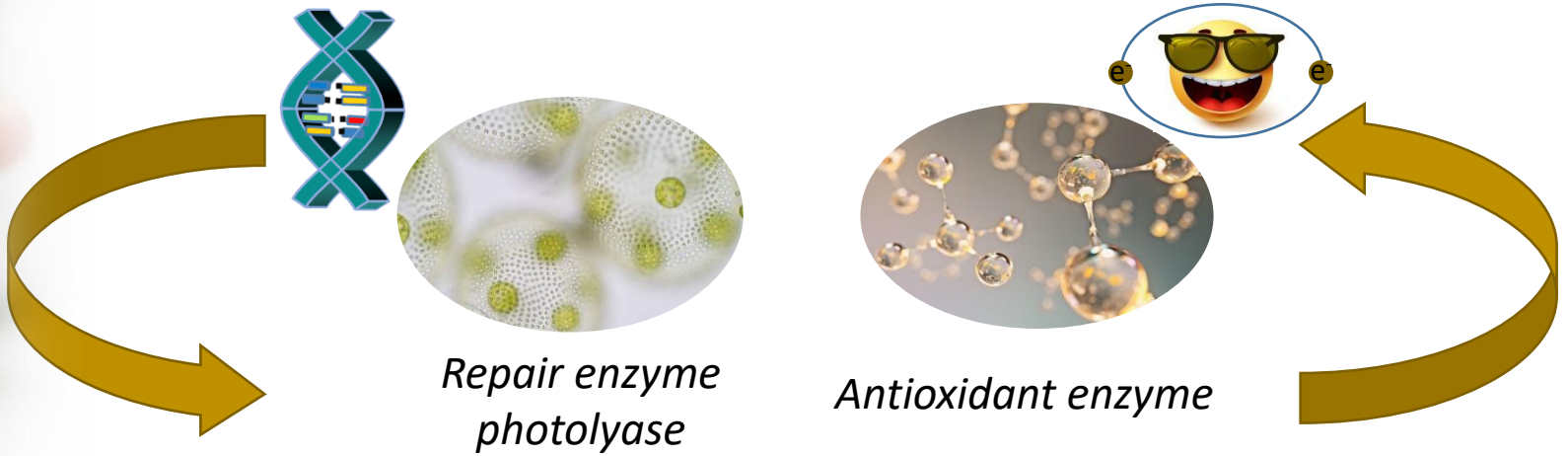
How stable is Fe(IV)?

Fe(IV) is a **very rarely** occurring oxidation state. This is due to its **generally low stability**.

Under certain conditions, however, this oxidation state can be **stabilised** and even be **impressively visible** to the naked eye: **Amethyst**

Amethyst consists of the mineral quartz. Its **violet colour** is caused by **iron impurities in the oxidation state Fe(IV)**.

Continuously Operating Partners: Repair Enzyme & Antioxidant Enzyme



Repair enzyme photolyase

Antioxidant enzyme

Both are not used up

- **Many repair cycles & continuous radical neutralisation possible**
- **Synergistic long-term repair & protection**

Photolyase: Gene Expression & Pathway Analysis

Differentially expressed genes of approx. 30,000 analysed genes:

a) With UVB irradiation, without photolyase treatment: 1874 genes
b) With UVB irradiation, with photolyase treatment: 788 genes

→ Photolyase directly or indirectly influences **1086 genes (58%)**, e.g.:

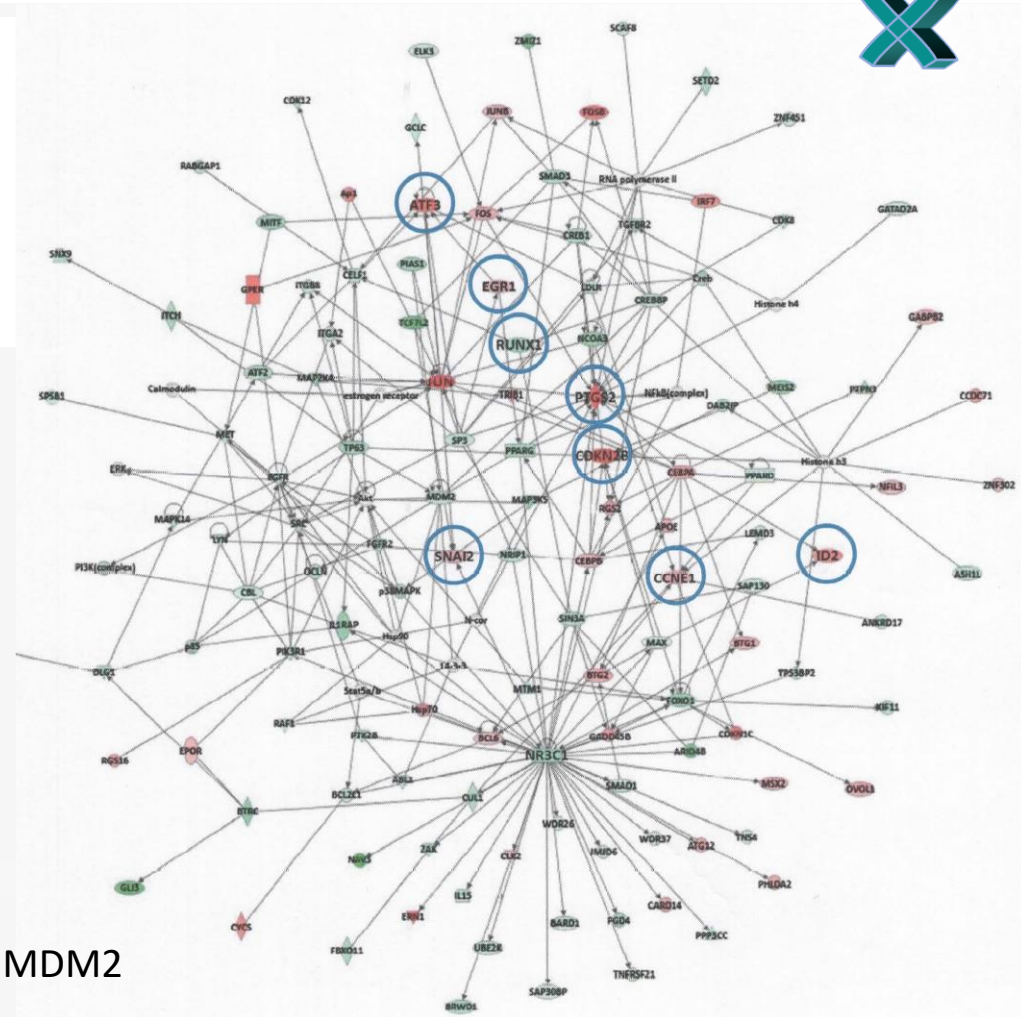
a) Cell cycle regulation
- up-regulated: Cyclin E1 (CCNE1), BTG 2, GADD45B
- down-regulated: CDK8 and 12, BARD1, ,SMAD3

b) Transcription factors
- up-regulated: SNAI2, MSX2, AFT3
- down-regulated: RUNX1, SMAD1, FOXO1

c) Apoptosis (cell death)
- up-regulated: BTG1, IL6, ERN1
- down-regulated: SCR, BCL2L11, FAF1

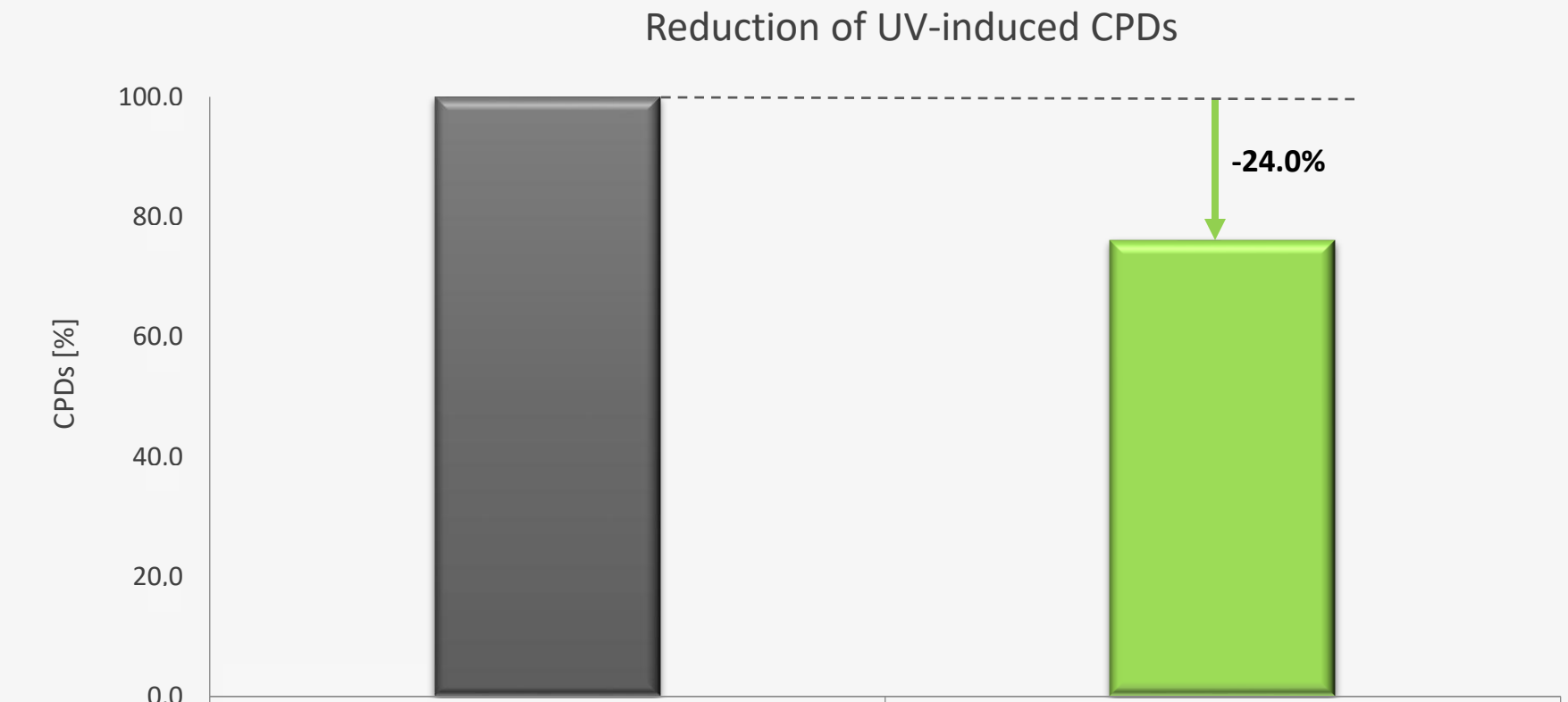
d) Cellular growth, proliferation
- up-regulated: PTGS2, EPOR, SNAI1
- down-regulated: IL15, PLD1, CDH13

e) Protooncogenes
- up-regulated: JUN, FOSB, JUNB
- down-regulated: EGFR, FGFR2, MDM2



Study design: Human 3D full-thickness skin models, irradiation: UVB radiation (220 mJ/cm2), subsequent treatment with photolyase (formulation used: aqueous active solution (with photolyase), placebo-controlled) + incubation for 24 h. ***Analysis****: Gene chip analysis (oligonucleotide-based DNA microarray analysis, in vitro), threshold: ± 2-fold gene expression compared to negative control (non-irradiated, untreated model).*

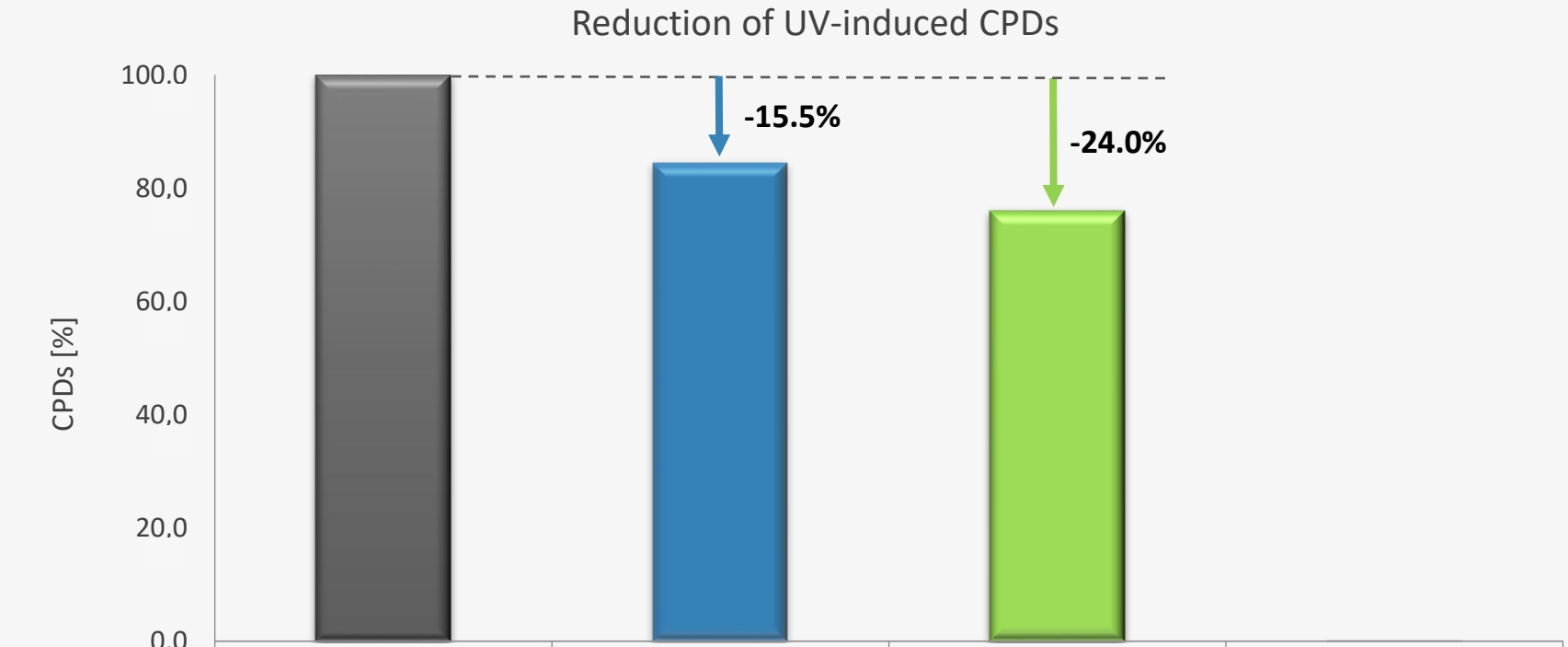
Efficacy Study 1a) – CPD Repair



Significant repair of UV-induced cell damage

Study design: Human 3D full-thickness skin models, used formulation: aqueous solution of active ingredient with 1% Glorydermal® Guard FS. Followed by Irradiation: UVB radiation (220 mJ/cm²), incubation for 24 h. Untreated, UV-irradiated = positive control (normalisation to 100%, maximum stress). Analysis: CPD ELISA assay (epidermal keratinocytes), results in relation to positive control ($p<0.01$).

Efficacy Study 1b) – CPD Repair & Synergism

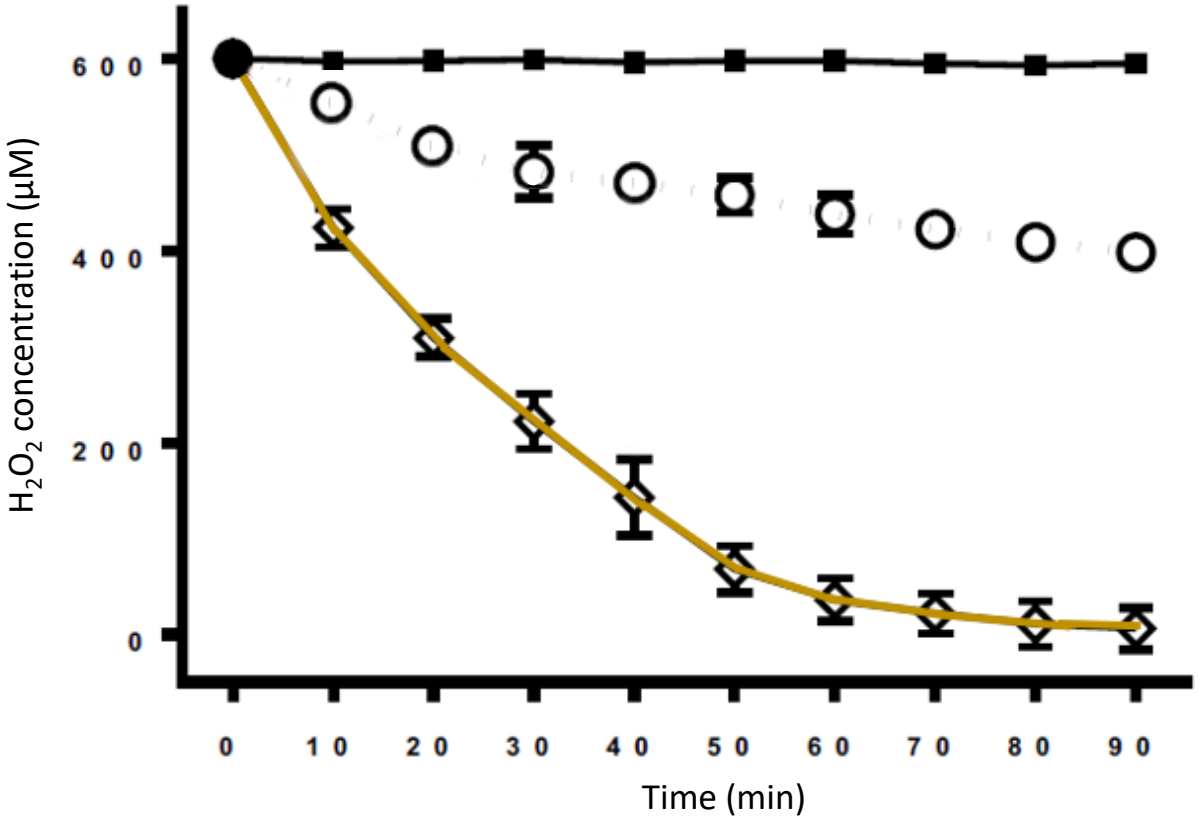


	Untreated, UV-irradiated (positive control)	Photolyase	Glorydermal® Guard FS	Untreated, non-irradiated (negative control)
CPDs after 24 h	100.0%	84.5%	76.0%	0.1%

Synergism: The antioxidant enzyme protects the CPD repair enzyme photolyase from oxidative degradation.

Study design: Human 3D full-thickness skin models, used formulation: aqueous solution of active ingredient with 1% Glorydermal® Guard FS or only with the corresponding photolyase concentration. Followed by Irradiation: UVB radiation (220 mJ/cm²), incubation for 24 h. Untreated, UV-irradiated = positive control (normalisation to 100%, maximum stress). Analysis: CPD ELISA assay (epidermal keratinocytes), results in relation to positive control ($p<0.01$).

Antioxidant Assay – Antioxidant Enzyme and Non-Enzymatic Antioxidant



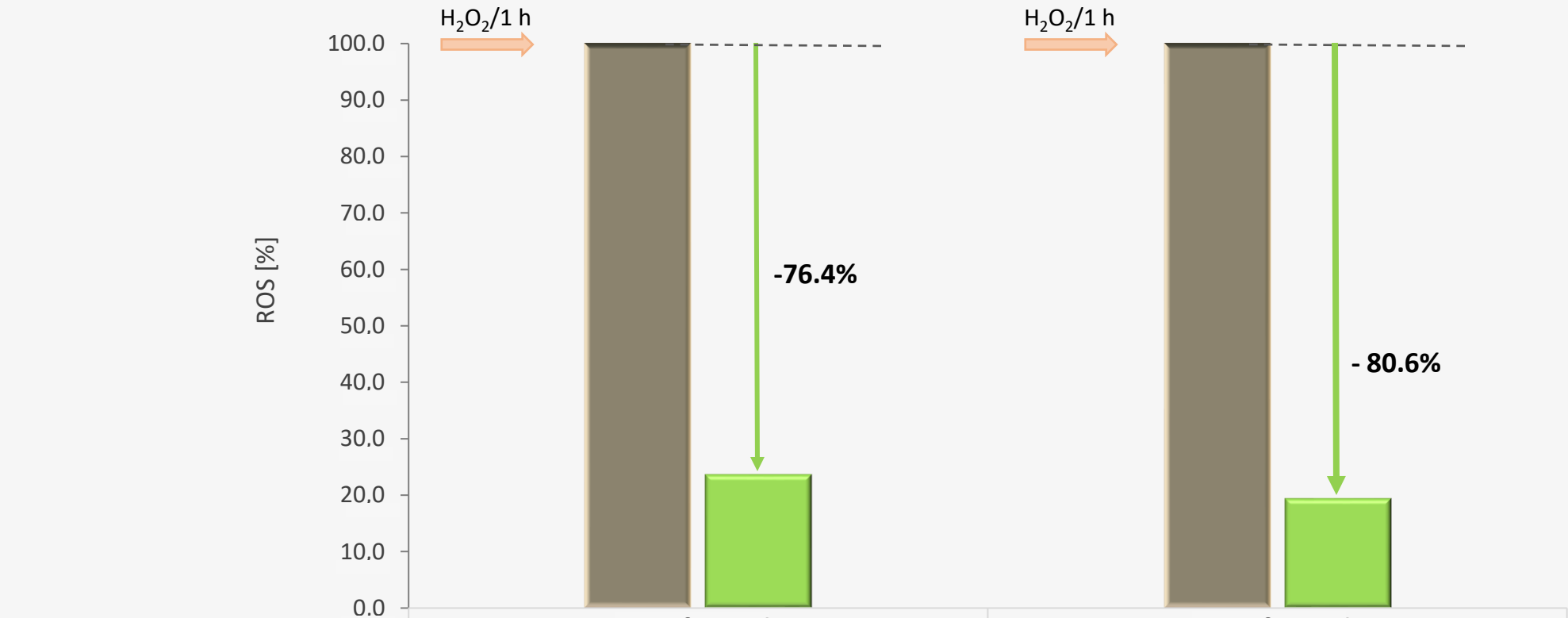
- Negative control, 100 µM
- Vitamin E (Trolox, hydrosoluble), 100 µM
- Iron peptide in Glorydermal® Guard, 10 nM

The rate of H_2O_2 breakdown for the **iron peptide** (antioxidant enzyme) is **significantly superior over time than the one for vitamin E** (non-enzymatic antioxidant), although its concentration is **10,000-times less** than the concentration of vitamin E.

→ **Proof of the enzymatic activity** of the iron peptide (one molecule can breakdown several H_2O_2 molecules), which enables long-term radical protection.

Assay design: Xylenol orange assay (A560) (Gay & Gebicki, 2000) to measure the H_2O_2 breakdown of antioxidant components over time.

Efficacy Study 2a) – Long-term Radical Protection

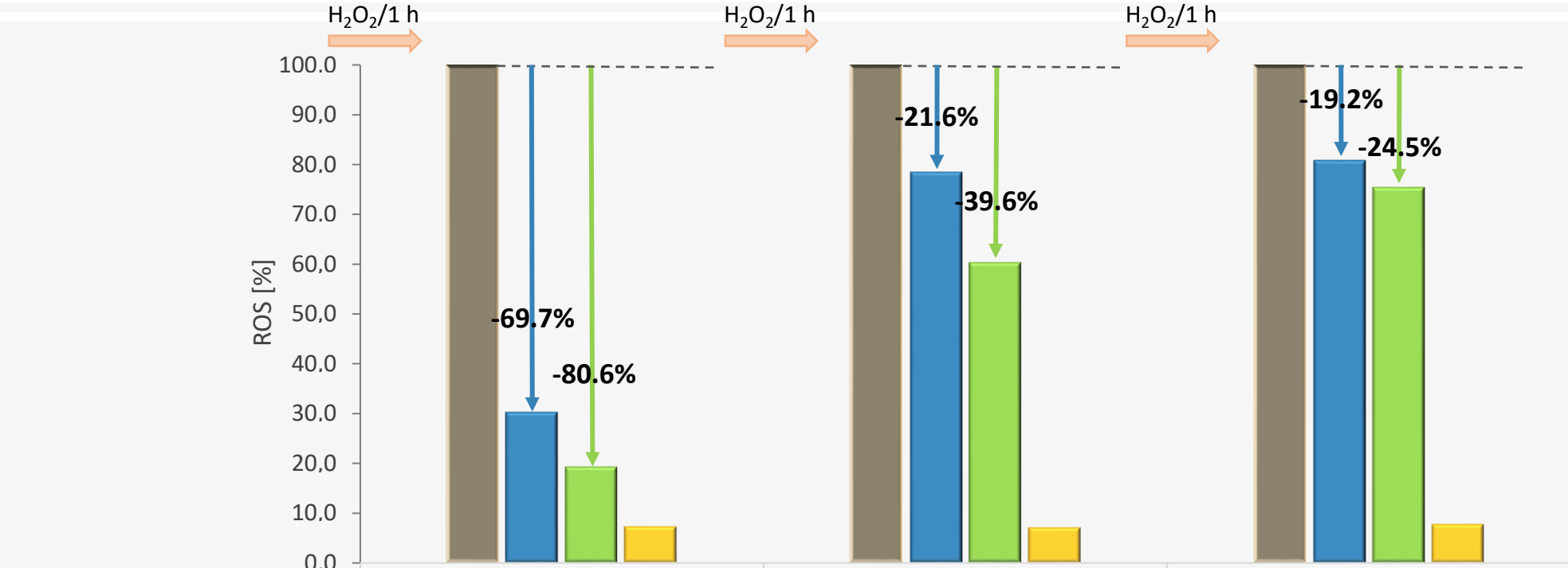


	ROS after 24 h	ROS after 48 h
untreated, ROS-induced	100.0%	100.0%
Antioxidant enzyme + Photolyase	23.6%	19.4%

The antioxidant enzyme regenerates itself and provides long-lasting protection against free radicals.

Study design: Human keratinocytes, used formulation: aqueous solution of active ingredient with 1% Glorydermal® Guard FS with respect to the antioxidant enzyme. ROS were induced by H_2O_2 treatment (treatment duration: 1 h). Untreated, ROS-induced = positive control (normalised to 100%, maximum stress). Results in relation to positive control ($p<0.0001$).

Efficacy Study 2b) – Long-term Radical Protection & Synergism



	ROS after 48 h	ROS after 72 h	ROS after 96 h
Untreated, ROS-induced	100.0%	100.0%	100.0%
Antioxidant enzyme	30.3%	78.4%	80.8%
Antioxidant enzyme + photolyase	19.4%	60.4%	75.5%
Untreated, not ROS-induced	7.3%	7.1%	7.8%

- The antioxidant enzyme regenerates itself and provides **long-term radical protection**.
- The photolyase supports the antioxidant enzyme **synergistically**.

Study design: Human keratinocytes, used formulation: aqueous solution of active ingredient with 1% Glorydermal® Guard FS with respect to the antioxidant enzyme or only with the corresponding concentration of the antioxidant enzyme. ROS were induced by H_2O_2 treatment (treatment duration: 1 h). Untreated, ROS-induced = positive control (normalised to 100%, maximum stress).
Untreated, not ROS-induced = intrinsic cell stress. Results in relation to positive control ($p<0.0001$).

In vivo Study – Antioxidant Protection Upon Oxidative Challenges

Biozoom® Skin Autofluorescence (Skin AF)

biozoom Services GmbH

Biozoom® measures **carotenoids** present in the skin by means of fluorescence techniques.

In use double blind, randomised, placebo-controlled

Hemiface application before exposure to oxidative challenges

A) Placebo cream vs verum cream (with 1% Glorydermal® Guard)

B) Placebo cream vs verum cream (with antioxidant enzyme corresponding to 1% Glorydermal® Guard)

5 women (A: aged 22-48 years, B: aged 25-32 years)

Exposure time of the different oxidative challenges: 15 min

Measurements at T0 and T1 (T0+15 min)

3 measurements in different areas/cheek for placebo and verum

Different oxidative challenges:

Ozone
(representing urban pollution)

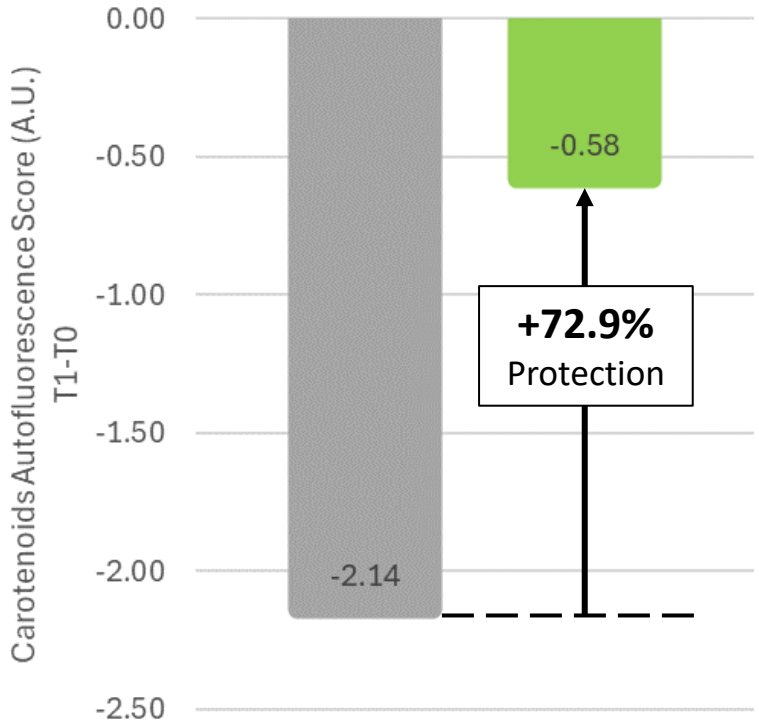
UVA
(380 nm)

HEV/Blue Light
(380-445 nm)

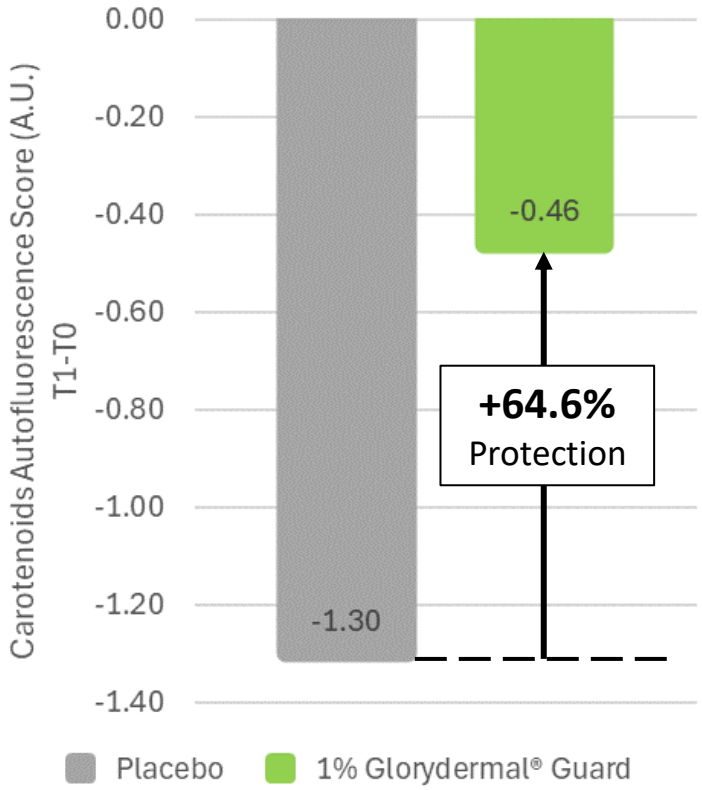
In vivo Study – Antioxidant Protection Upon Oxidative Challenges (A)

The less oxidation of carotenoids (smaller bar) vs placebo, the higher the antioxidant protection.

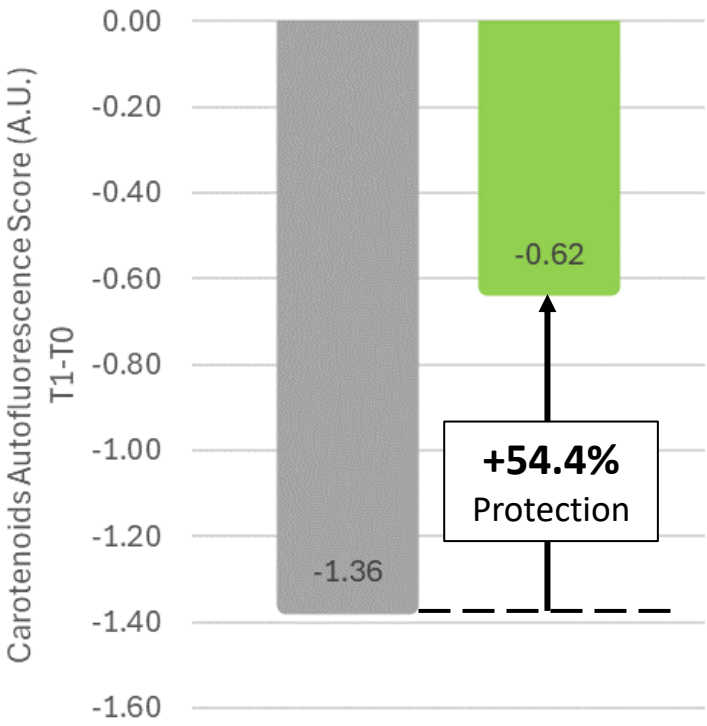
Ozone (representing urban pollution)



UVA (380 nm)



HEV/Blue Light (380-445 nm)

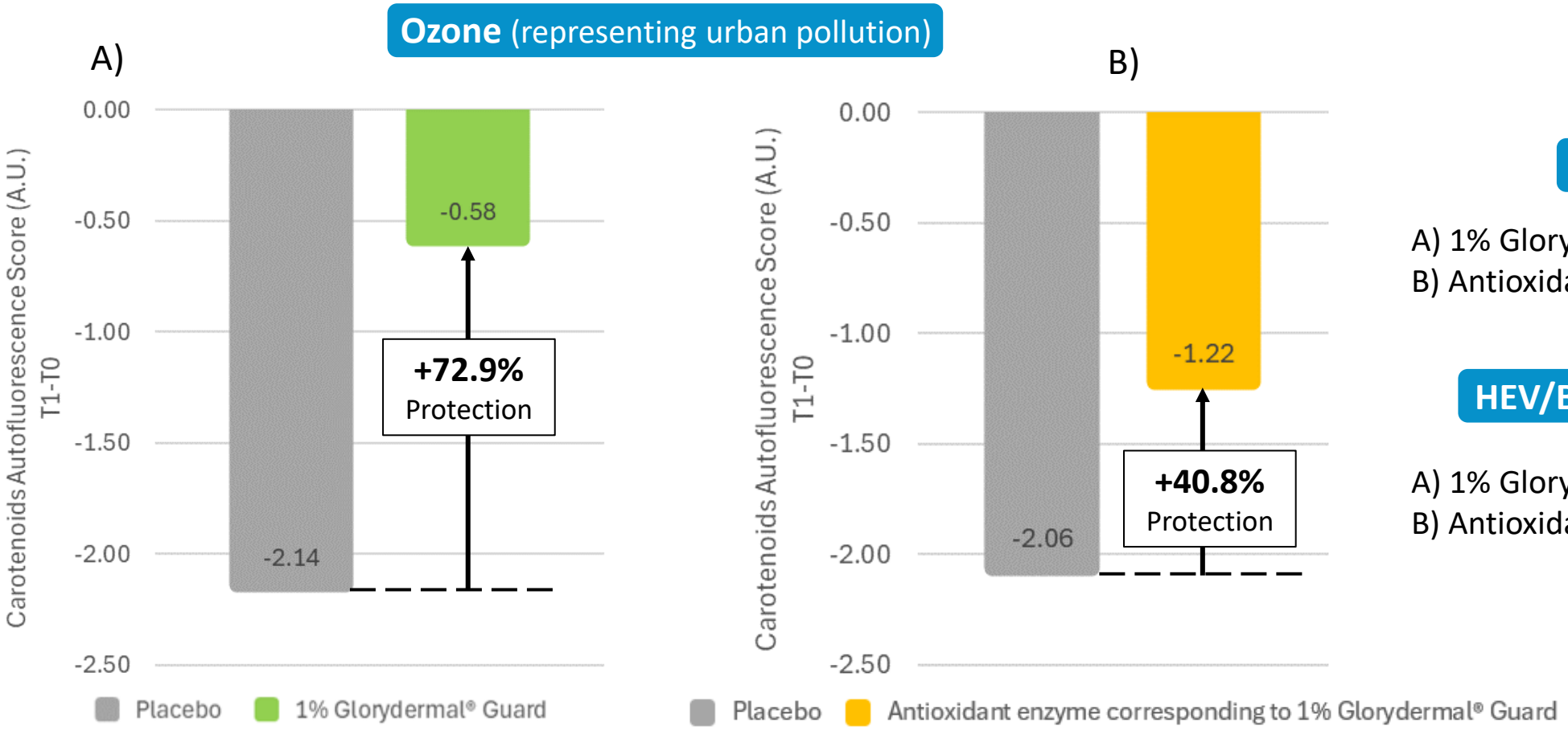


Strong antioxidant power: after 15 min exposure to the corresponding oxidative challenge, **less carotenoids were oxidised** in the skin in all cases **with Glorydermal® Guard**.

In vivo Study – Antioxidant Protection Upon Oxidative Challenges (A+B)

The less oxidation of carotenoids (smaller bar) vs placebo, the higher the antioxidant protection.

Ozone (representing urban pollution)



UVA (380 nm)

A) 1% Glorydermal® Guard: **+64.6%**
B) Antioxidant enzyme alone: **+39.3%**

HEV/Blue Light (380-445 nm)

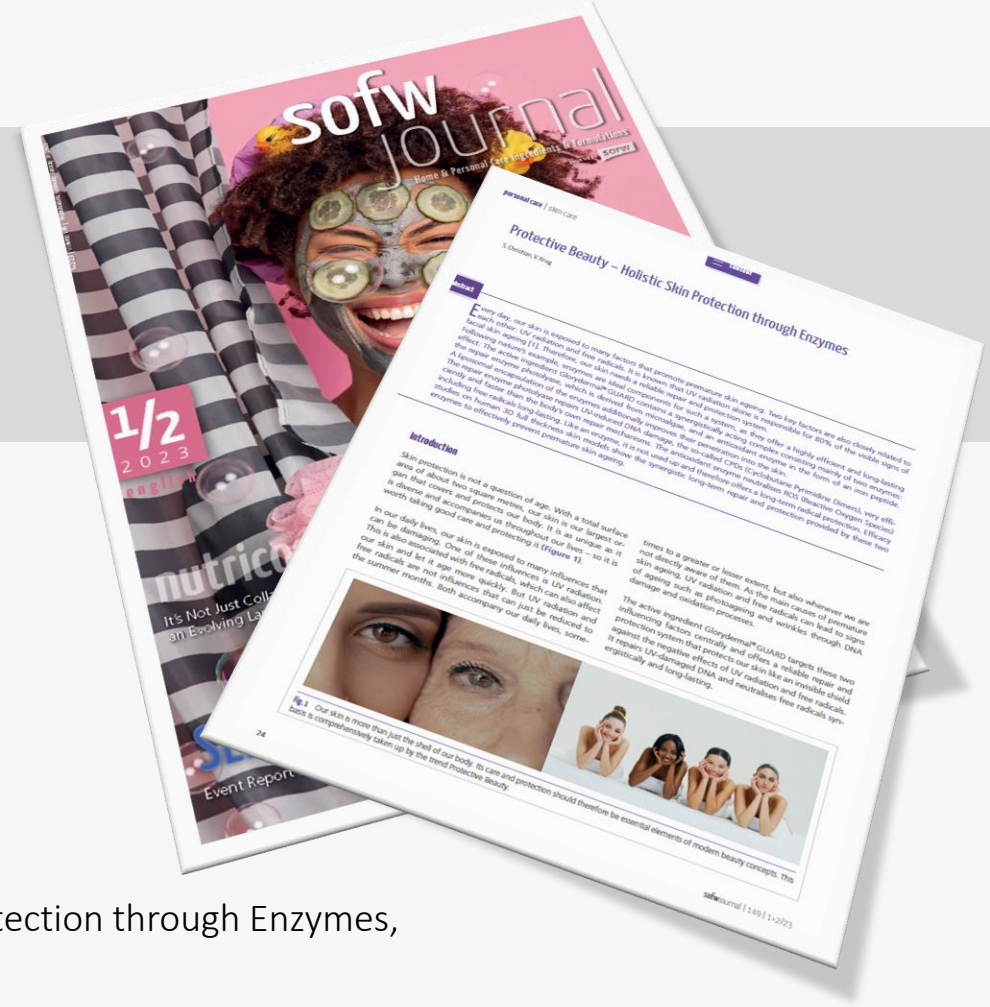
A) 1% Glorydermal® Guard: **+54.4%**
B) Antioxidant enzyme alone: **+39.1%**

Strong synergistic effect: after 15 min exposure to the corresponding oxidative challenge, the **combination of the antioxidant enzyme with the repair enzyme photolyase** in Glorydermal® Guard offers a **higher antioxidant protection** than the antioxidant enzyme alone.

SOFW JOURNAL – Best Paper Award 2023

2nd prize

Submission of 51 articles



S. Christian, V. Krug, Protective Beauty – Holistic Skin Protection through Enzymes, *SOFW Journal* **2023**, *149 (1+2/23)*, 24-30.

Glorydermal® Guard

Product Code: GD-GG-003

INCI EU (CTFA/PCPC):
AQUA (WATER), PLANKTON EXTRACT, LECITHIN, GLYCERIN, XANTHAN GUM, HEMIN PENTAPEPTIDE-128 GAMMA-GLUTAMYL DIPEPTIDE-4.

ADDITIVES, PRESERVATIVES:
PROPANEDIOL, 1,2-HEXANEDIOL, CAPRYLYL GLYCOL, LACTIC ACID.

INCI CHINA (IECIC (I)) is available separately.

Appearance: light yellow to light grey, hazy liquid

Solubility: dispersible in water

Recommended dosage: 1%

Formulation: at the end of the production at a temperature < 40°C

VEGAN

COMPLIANT

Summary – LONGEVITY & PROTECTIVE BEAUTY

- Comprehensive, reliable skin protection without burdening
- Synergistic long-term effect through repair enzyme photolyase and antioxidant enzyme
- Continuous repair of DNA damage and long-lasting neutralisation of ROS (Reactive Oxygen Species, free radicals)
- Easy handling in the production of cosmetic products (addition at the end of the production, cooled bulk)
- Recommended for daily care (serum, cream, etc.), body care, sunscreen and after sun products

Glorydermal® Guard

GloryDermal

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