

Skin proteome protection, a new ecobiological target in healthy aging

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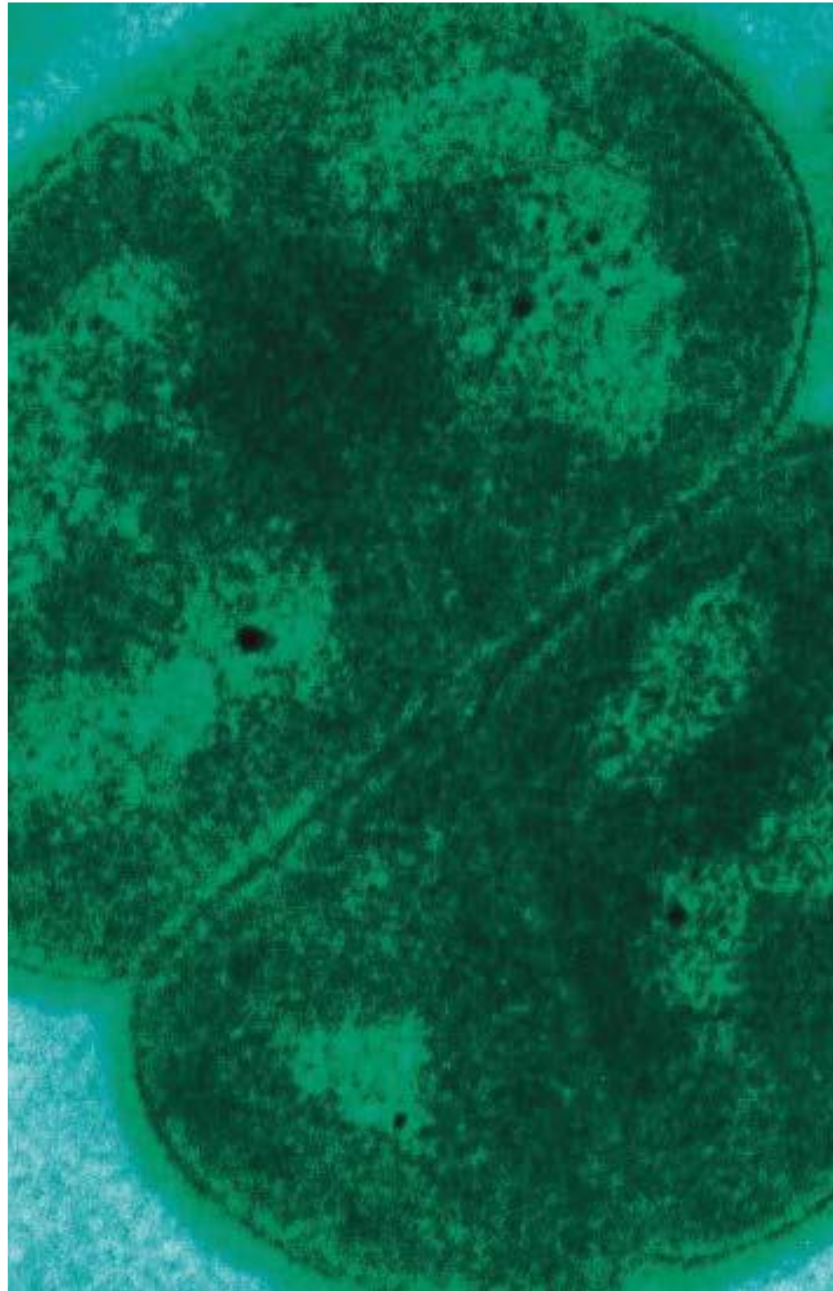
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INTRODUCTION



- * *Deinococcus radiodurans* bacteria are a model to study longevity as they can survive in extreme environmental conditions. Their analysis led to a new scientific paradigm: protection of the proteome is the key to cellular longevity. Indeed, their DNA is altered but repaired by ultra-resistant proteins protected by molecules with chaperone-like and antioxidant activities. In skin, among the most harmful damages, **carbonylation**, an irreversible alteration specific to proteins, generates toxic aggregates for the cells, **leading to premature aging**.
- * The aim of this study was to evaluate the efficacy of **bacterioruberins**, a new class of **chaperone-like and antioxidant molecules** extracted from another extremophilic bacteria, *Arthrobacter agilis*, on proteome protection and consecutive prevention of all skin aging signs.

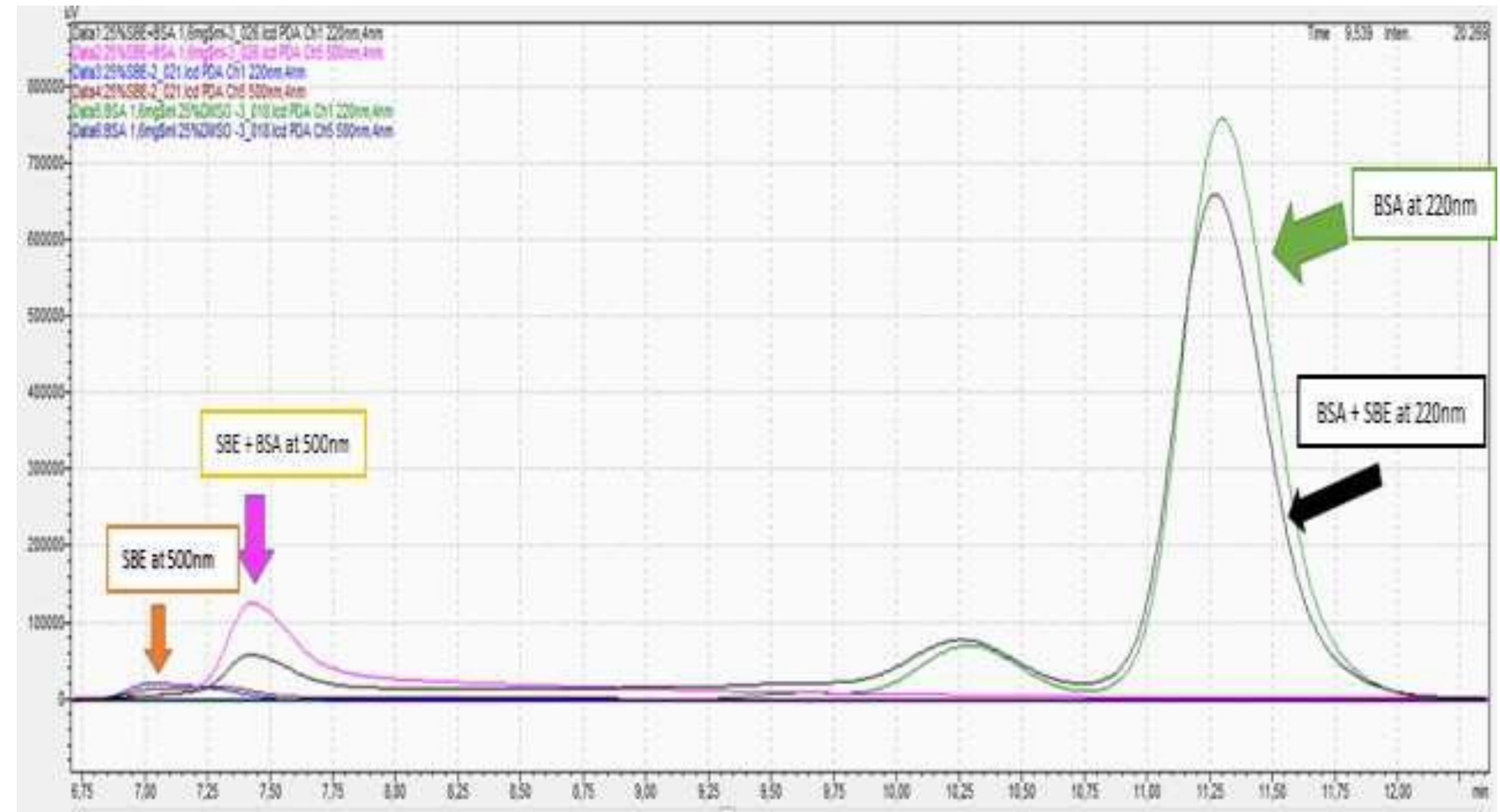
MATERIALS & METHODS

- First, we assessed *in tubo* bacterioruberins on the **physical protection** *via* chaperone-like effect using both the Bovine Serum Albumin (BSA) protein test with HPLC analysis (to assess protein binding capacity) and the Alkaline Phosphatase (AP) heat test at 55°C, and on the **biological protection**, *via* antioxidant properties using ABTS test. All tests assessed reference molecules.
- Second, **evaluation of carbonylated protein level** was performed:
 - ***in vitro*** on normal human keratinocytes exposed to UVA (365nm, 28J/cm²), fine particles (PM10 50µg/mL) or blue light (460nm, 72J/cm²) using a specific fluorescent probe targeting carbonyls products;
 - ***in vivo*** during a **28-day clinical study** on 23 smoking women (38 to 69 years old), phototypes II to III with a dull complexion, applying on hemi-face twice daily a serum containing bacterioruberins versus placebo. A carbonylation score was assessed by measuring the total proteins (Bradford methods) and the carbonylated protein (specific fluorescent probe) on protein extracted from D-Squam samples performed on the hemi-faces.
- Finally, a **clinical study** was performed on 55 women (42 to 65 years old) who applied for 6 months twice daily on half-face a **neutral cream and a serum containing bacterioruberins vs. the neutral cream alone**. Each month, the main aging signs were scored (wrinkles [crow's feet, under-eye and nasolabial fold], firmness, density, complexion, pigment spots and radiance) from which a global aging evolution was calculated, and pictures were taken.

CHAPERONE-LIKE ACTIVITY *in tubo* (1/2)

- * Maximum absorbance of bacterioruberins (SBE) is at 500 nm, and of BSA is at 220 nm, thus generating two very distinct profiles.
- * Thanks to the different profiles established and by studying the BSA + bacterioruberins' profile at 220 nm (black curve), we can see that the presence of bacterioruberins with BSA modifies its profile.
- * Under these conditions, we observe that the peak at 220 nm was slightly shifted to the left, indicative of a faster elution and therefore associated with a heavier molecule due to physical interaction between these two molecules. At 500 nm, the peak of bacterioruberins (SBE) alone appeared earlier than bacterioruberins + BSA, maybe due to a steric hindrance .

Bacterioruberins physically interacted with BSA protein

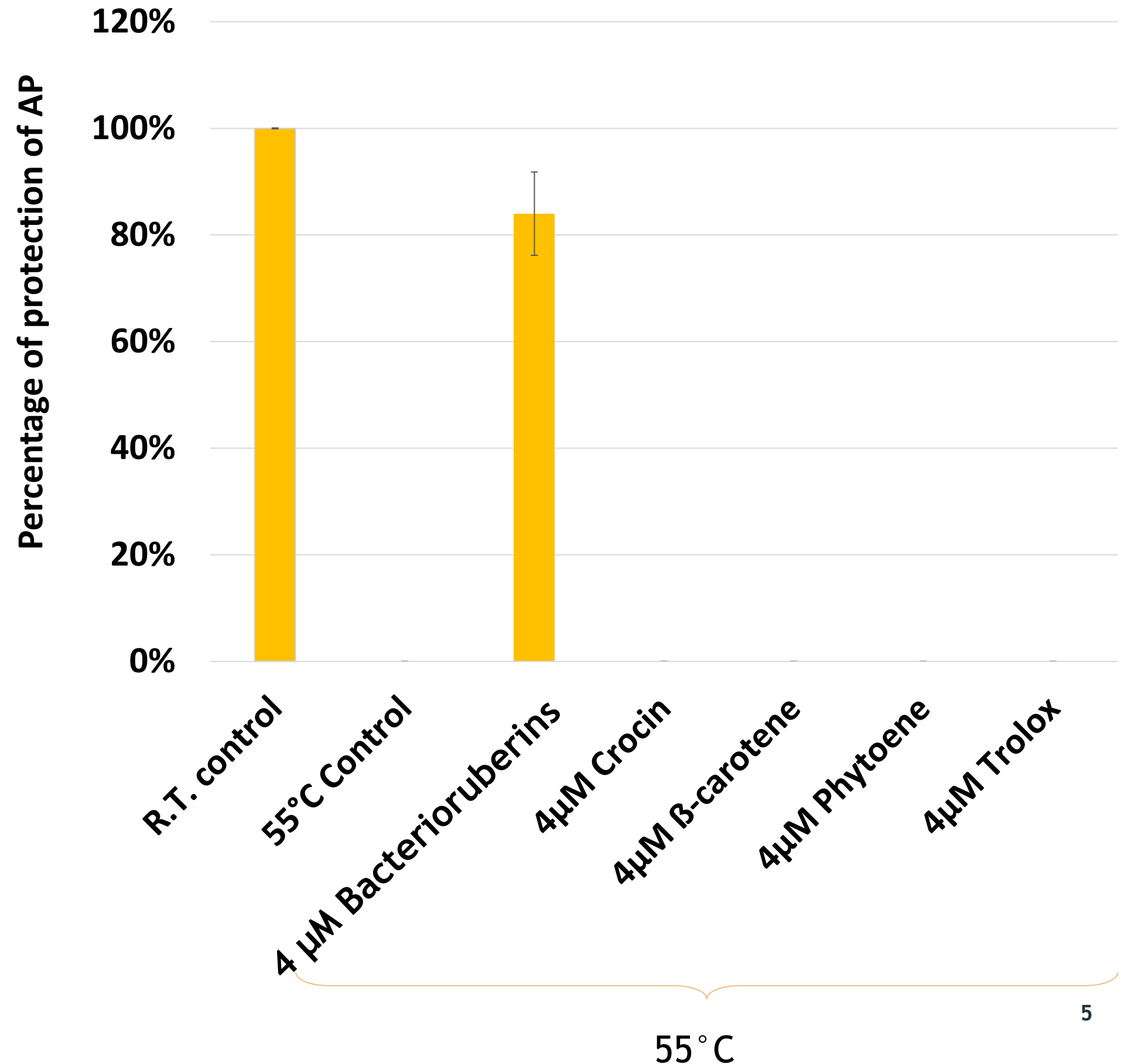


Legend: *bacterioruberins at 220nm* / *bacterioruberins alone at 500nm* / *BSA alone at 220nm* / *BSA alone at 500nm* / *bacteriorubrins + BSA at 220nm* / *bacterioruberins + BSA at 500nm*

CHAPERONE-LIKE ACTIVITY *in tubo* (2/2)

- * The measured Alkaline Phosphatase (AP) activity was inhibited after the heat stress (55° C control versus R.T. control)
- * Among all the molecules tested, only bacterioruberins showed a protein protection through chaperone-like effect, with more than 80% of AP activity protection at 4µM.
- * By binding to and protecting proteins, bacterioruberins display chaperone-like activity.

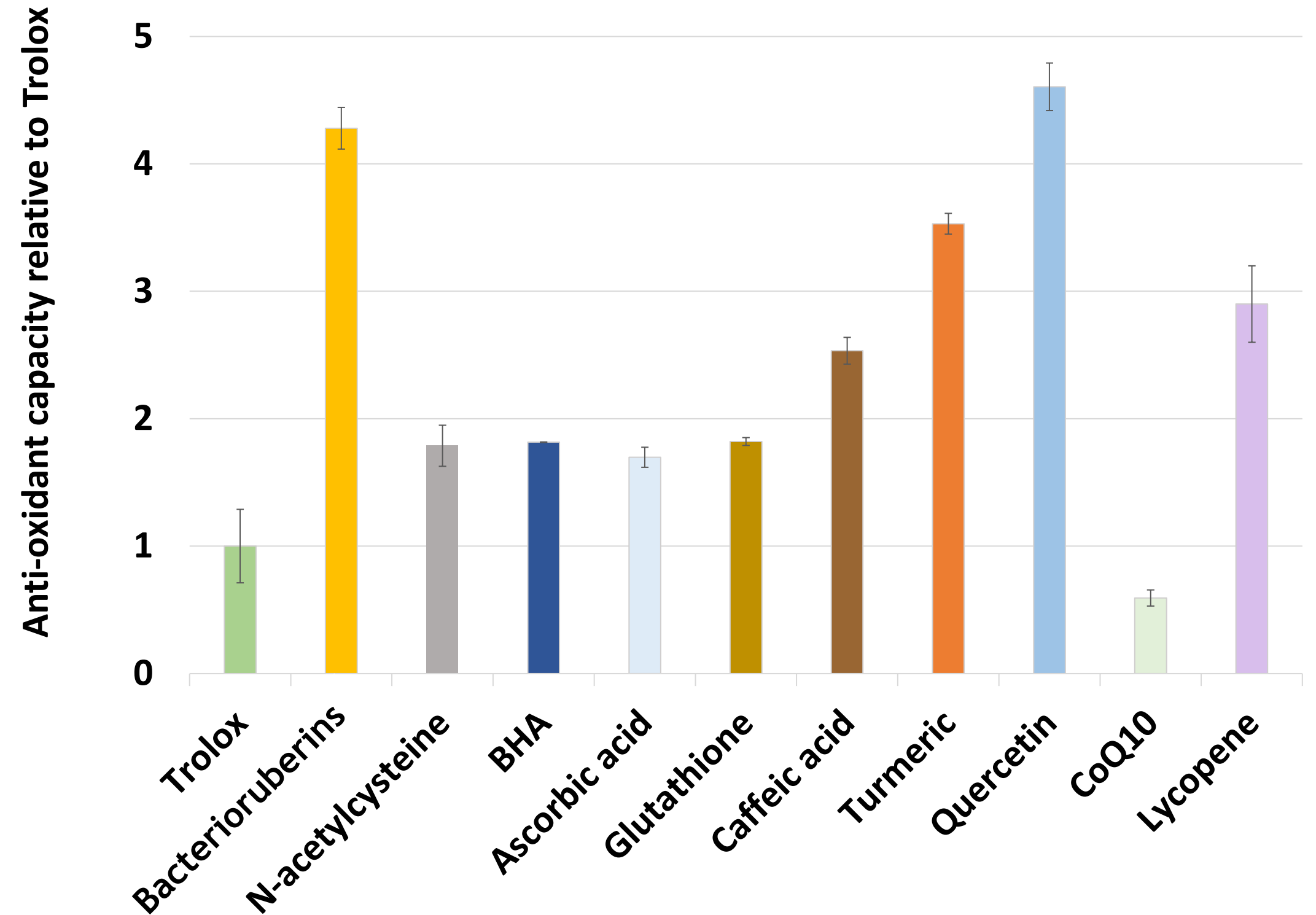
Bacterioruberins protected Alkaline Phosphatase protein from heat



ANTIOXIDANT POWER *in tubo*

- * Moreover, in the ABTS test bacterioruberins presented a stronger antioxidant potential than reference antioxidants, comparable to that of quercetin, corresponding to 4 times more than Trolox's one (vitamin E derivate).

Bacterioruberins had a strong antioxidant potential, higher than reference antioxidant molecules

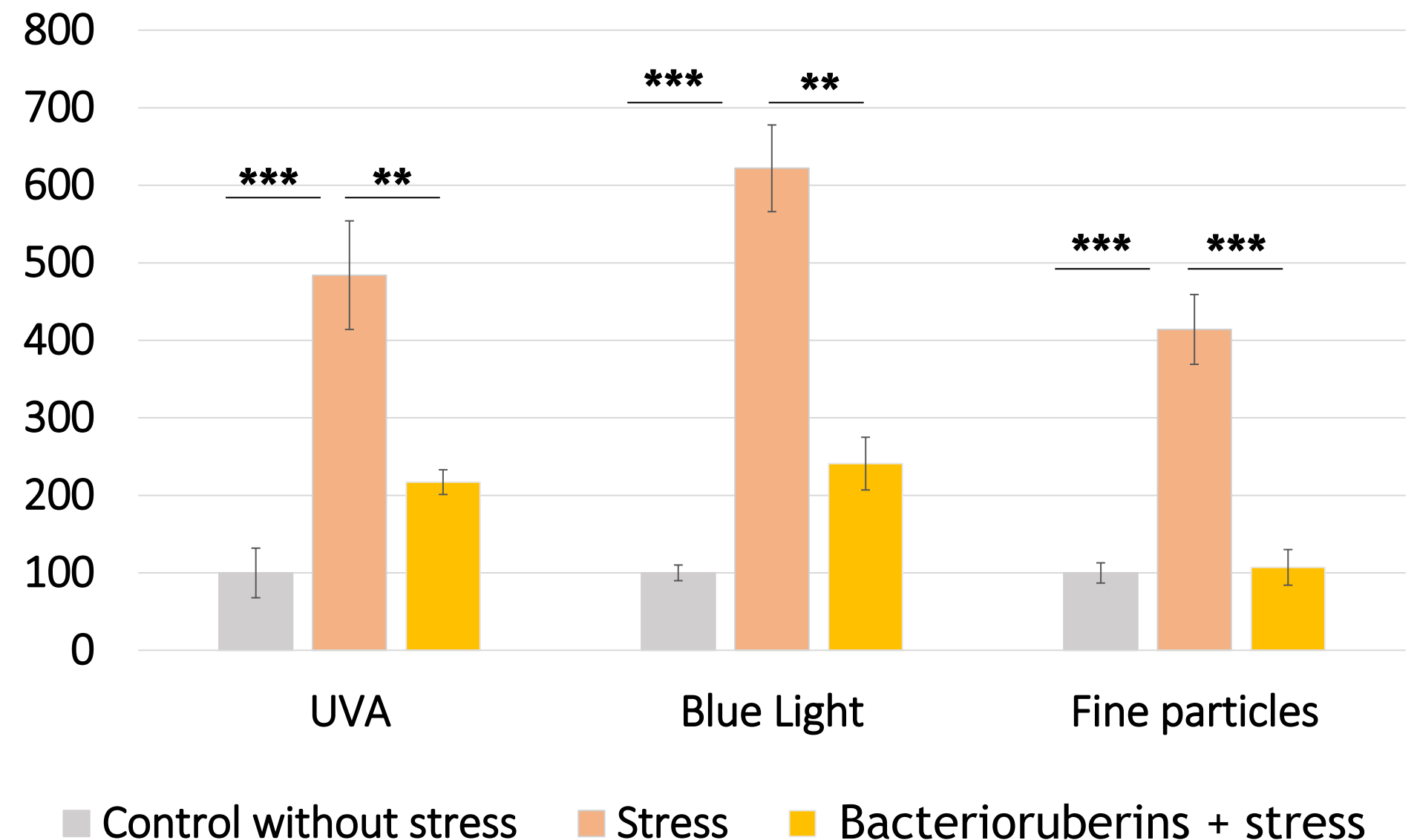


PROTECTION AGAINST CARBONYLATION *in vitro*

- * The three stresses (UVA, blue light and fine particles) significantly increased carbonylation within normal human keratinocytes.
- * Furthermore, bacterioruberins prevented 70% of protein carbonylation after exposure to UVA or blue light, and 100% after exposure to fine particles (pollution).

Intensity of fluorescence normalised to the number of cells and reported as percentage of control

Bacterioruberins protected against carbonylation induced by different stresses *in vitro*

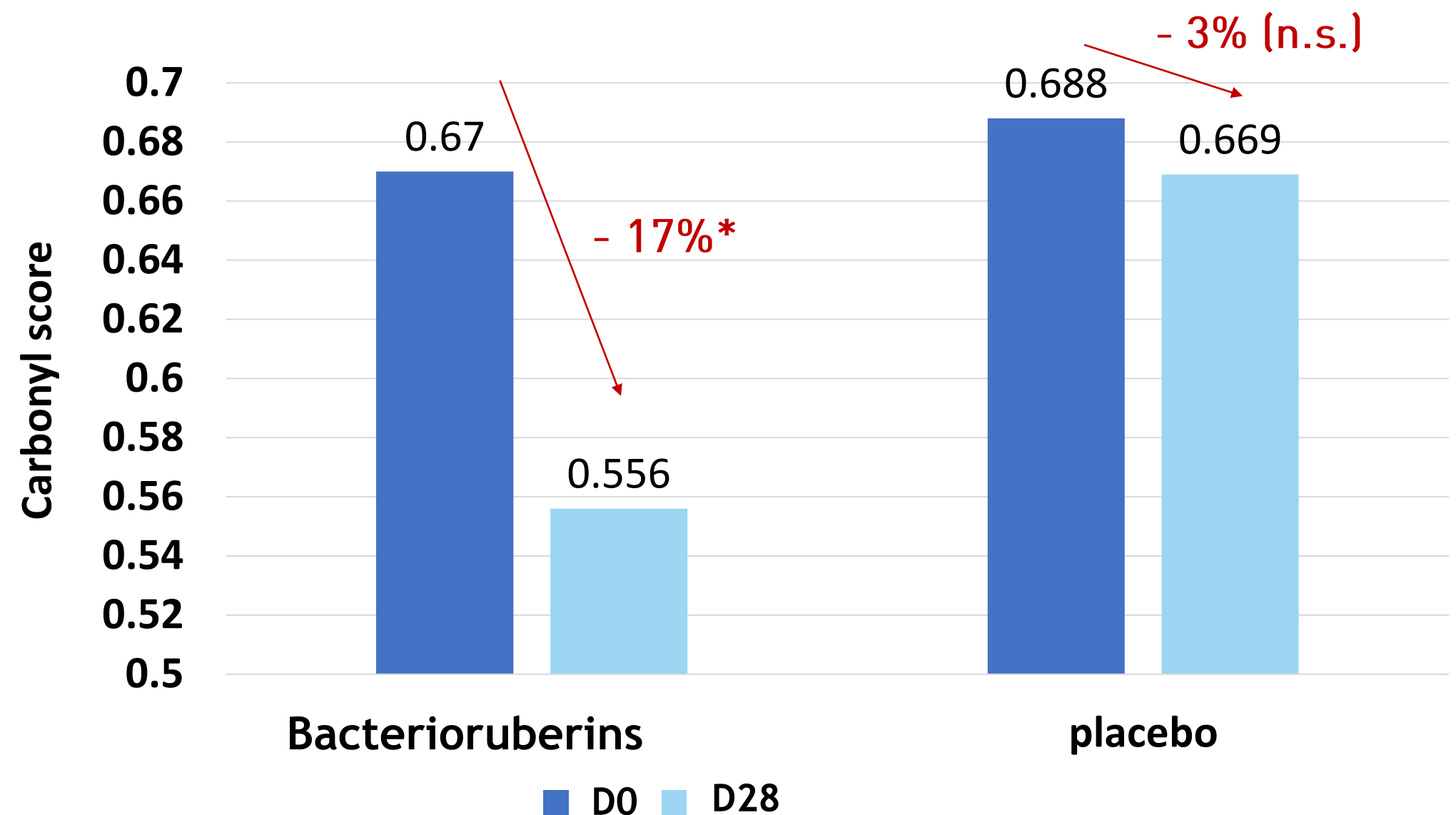


p<0.001; *p<0.001; ANOVA Bonferroni's Multiple Comparison Test

PROTECTION AGAINST CARBONYLATION *in vivo*

* The use of the serum with bacterioruberins induced a statistically significant decrease in the carbonylation rate of 17% in the *stratum corneum* at D28 compared to D0 (p<0.05, paired Student t-test).

Bacterioruberins reduced the level of protein carbonylation compared to placebo



*p<0.05, Student t Test

REDUCTION OF SKIN AGING SIGNS *in vivo*

* Skin tone and radiance



* Pigment spots



* Wrinkles



REDUCTION OF SKIN AGING SIGNS *in vivo*

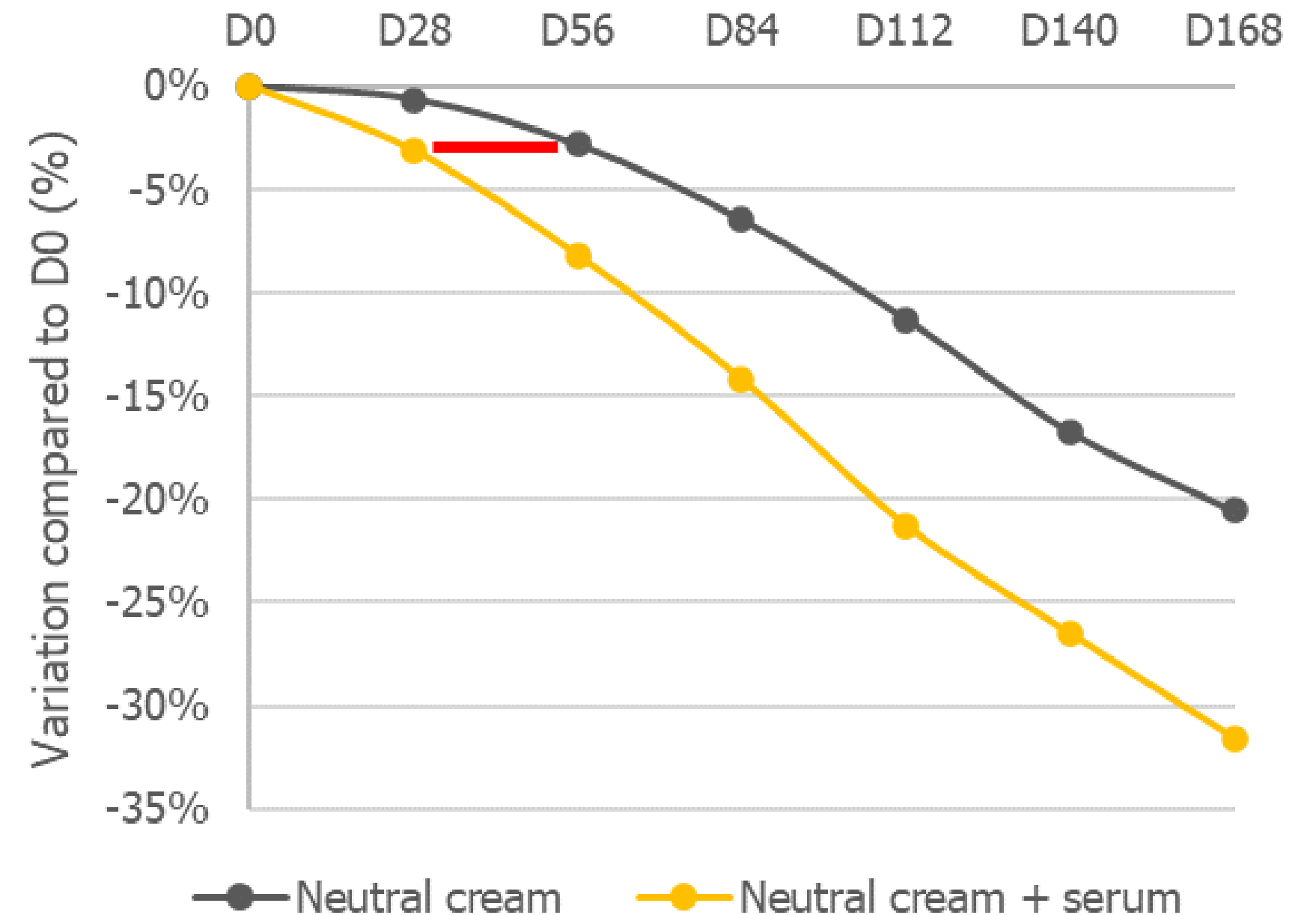


- * We used the results of the clinical scoring to calculate a clinical global aging evolution for each month on each hemi-face :

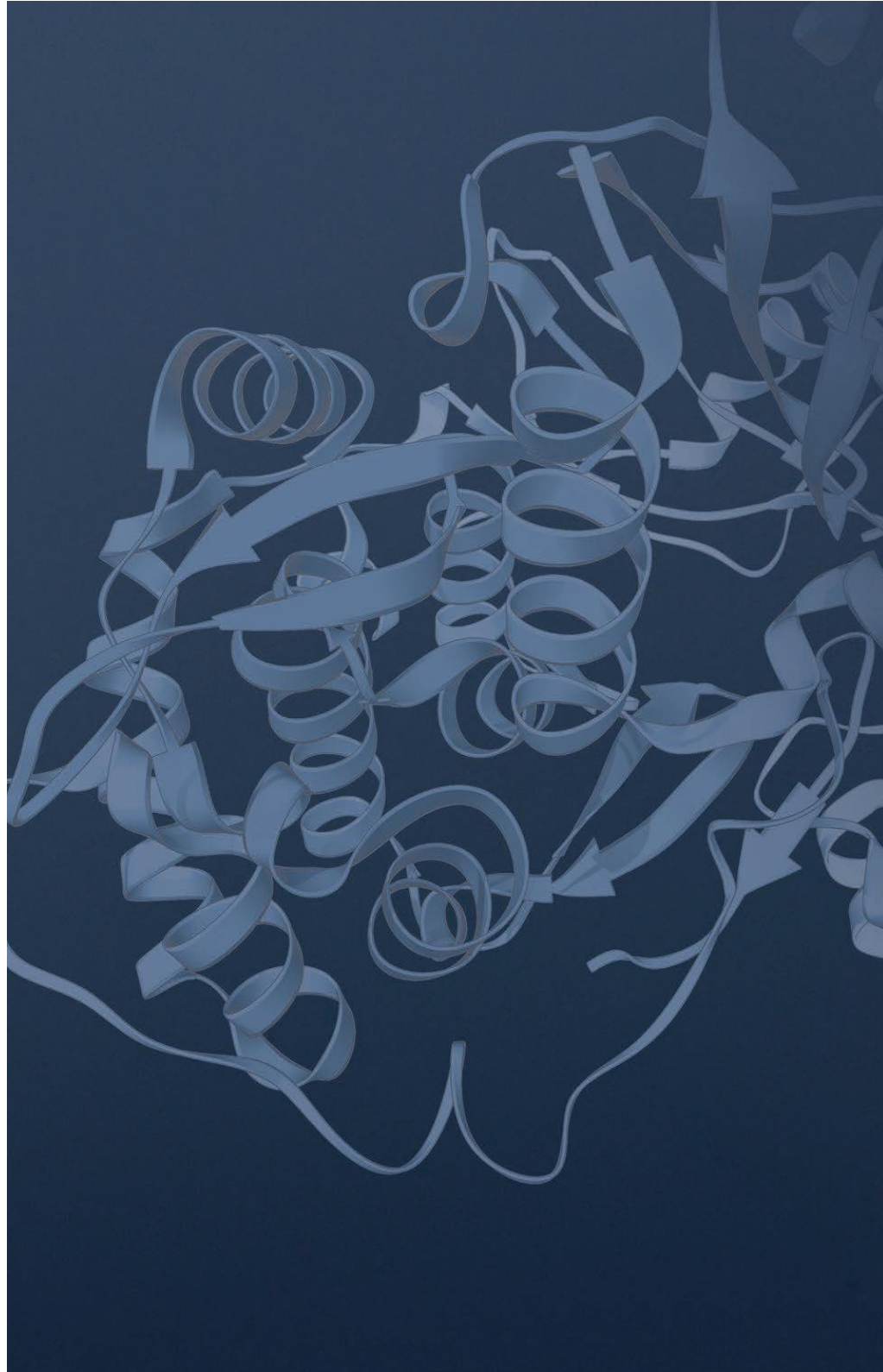
$$\begin{aligned} &= (\text{scores [wrinkles in crow's feet area]} + \text{score [wrinkles in under-eye area]} + \text{score [nasolabial fold]}) / 3 \\ &\quad + \text{score [loss of firmness]} + \text{score [loss of density]} + \\ &(\text{score [complexion homogeneity]} + \text{score [pigment spots appearance]}) / 2 \\ &\quad + \text{score [loss of skin radiance]} \end{aligned}$$

- * The clinical global aging evolution measured on the serum-treated side showed a visible and significant decrease of skin aging compared to D0 as early as 1 month, with a **decrease variation up to 31.6% after 6 months of application.**
- * Moreover, compared to the side treated with the neutral cream alone (placebo), the serum containing bacterioruberins improved the global aging 55% faster after 1 month when compared to the placebo after 2 months.
- * No adverse effect of the serum was observed and the galenic was well appreciated and tolerated.

Bacterioruberins reduced the global aging evolution



CONCLUSION



- * Proteome protection is key for cellular longevity. Thanks to the chaperone-like and antioxidant properties of bacterioruberins contained in a serum, the latter induces visibly higher and faster improvements of all aging signs.

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