UNIVERSITÀ DEGLI STUDI DI MILANO
Istituto di Chimica Farmaceutica e Tossicologica P. Pratesi

Congresso 32° Nazionale SICC

Marina Carini

IL VENTENNIO DI RICERCA DEI "MILANESI" E PROSPETTIVE PER I PROSSIMI 20 ANNI

Milano, 16 novembre 2006

1974

R. Maffei Facino

1995

G. Aldini

1983

M. Carini

2003

M. Orioli

2006

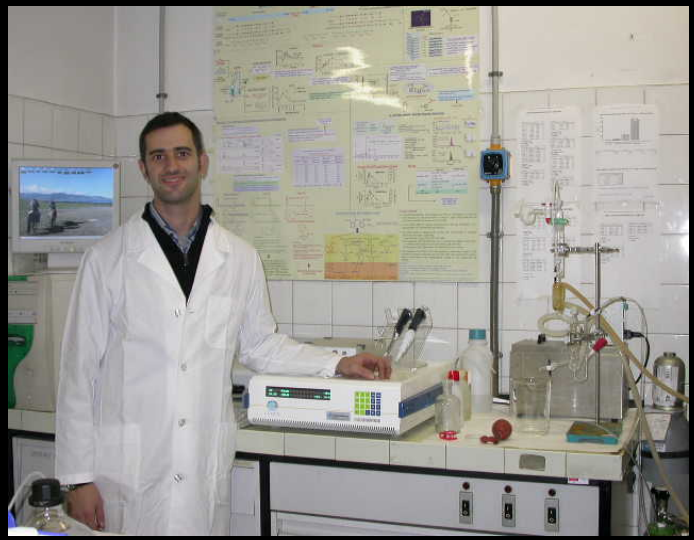
G. Beretta

Spettrometria di massa

Dr. Luca Gamberoni

Analisi cromatografiche

Dr. Luca Regazzoni



Chemiluminescenza

Dr. Fabrizio Gelmini

Biologia cellulare

Sig.ra Cristina Marinello

Analisi biochimiche

Dr.ssa Maria Benfatto

Dr.ssa Marina Zarrella

14th IFSCC Barcelona 1986

Efficacy of topically applied *Hedera helix L.* saponins for treatment of liposclerosis

R. Maffei Facino et al. Acta Ther. (1990)

Isolamento, caratterizzazione e valutazione attività biologica di prodotti naturali → studi *in vivo*

Antierythematous and photoprotective activities in guinea pigs and in man of topically applied flavonoids from *Helichrysum italicum G. Don.*

Crude extract application post-irradiation (3 MED)

↓

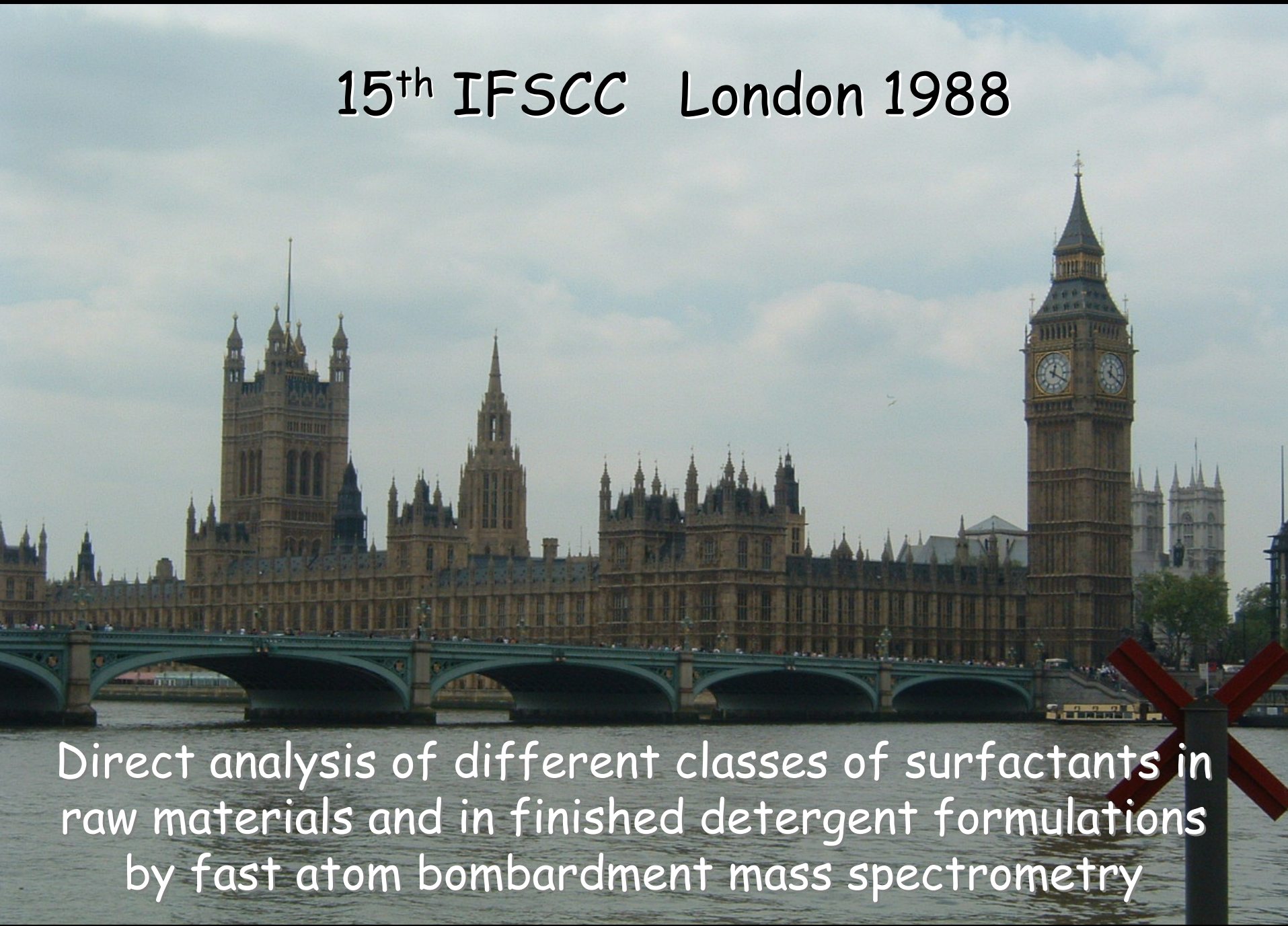
70% decrease of the erythematous response at 24h

extract application pre-irradiation

↓

SPF 5.2

R. Maffei Facino et al. Acta Ther. (1988)


15th IFSCC London 1988
Direct analysis of different classes of surfactants in raw materials and in finished detergent formulations by fast atom bombardment mass spectrometry

La spettrometria di massa per la definizione della composizione

ingredienti cosmetici

Tensioattivi
Conservanti
Saponine (*Hedera helix*)
(*Escina*)
Caffeoil derivati
(*Echinacea*)
Flavonoidi
Polifenoli (*Krameria triandra)*
(Ilex paraguayensis)
(Helichrysum)

prodotti finiti

Shampoos
Mascara
Anticellulite
Deodoranti
Tinture per capelli

Biomed. Environ. Mass Spectrom. (1987; 1988; 1989; 1990); *Org. Mass Spectrom.* 1991; *JAOCS* (1995); *Rapid Commun. Mass Spectrom.* 1997

SPETTROMETRIA DI MASSA

Anni 70-80

Nuove sorgenti
e nuovi sistemi
di ionizzazione

Anni 90

Analisi di molecole
di piccole dimensioni

Analisi di
macromolecole

Termolabili – ioniche – elevato P.M.

Isolamento, caratterizzazionen e valutazione attività biologica di prodotti naturali → studi *in vitro*

Antihyaluronidase and antielastase activity of saponins from *Hedera helix*, *Aesculus hippocastanum*, and *Ruscus Aculeatus* : an explanation for their efficacy in the cosmetic treatment of liposclerosis

Phytochemical characterization and radical scavenger activity of flavonoids from *Helichrysum italicum G. Don* and *H. stoechas*

LC-MS screening of phenolic antioxidants of Matè *(Ilex paraguayensis)*

LC-MS screening of phenolic constituents with radical scavenging activity from *Krameria triandra* roots.

Pharmac. Res. 1990; *Arch. Pharm.* 1995;
Rapid Commun. Mass Spectrom. 1997 + 1998; *J. Pharm. Biomed. Anal.* 2001;

Echinacoside and caffeoyl conjugates protect collagen from free radical-induced degradation: a potential use of *Echinacea* extracts in the prevention of skin photo-damage

R. Maffei Facino et al. Planta Med. 1995

Characterization of caffeoyl esters with antihyaluronidase activity in crude extracts from *Echinacea angustifolia* roots

R. Maffei Facino et al. Il Farmaco 1993

Attività radical scavenging: modelli cell-free

Radicale DPPH: capacità H transferring → cinetica UV

Anione superossido: metodi alternativi al sistema
xantina/xantina ossidasi
analisi EPR

Radicale OH°: sonolisi H_2O e analisi EPR

Radicale ROO°: azo iniziatori e analisi fluorimetrica
(ORAC assay)

Rigenerazione Toc-OH: formazione Toc-O° (UVB)
monitoraggio EPR

R. Maffei Facino et al. Rapid Commun. Mass Spectrom (1996)

Attività radical scavenging: modelli cell-free

Perossidazione lipidica

Substrato lipidico

Membrane artificiali (liposomi PC)

Micelle di acidi grassi poliinsaturi

Membrane microsomiali

Membrane eritrocitarie

Induttore

ADP/NADPH/Fe2+

Azo iniziatori

Idroperossidi organici

Sonolisi

UVB

Biomarker

- End point-markers di danno di membrana

  TBAR – MDA - Dieni coniugati - 2,4-dinitrofenilidrazoni

- Markers di danno precoce → Probes fluorescenti

Attività radical scavenging: modelli cellulari

Induttori chimici/fisici

❶ eritrociti (RBC)
❷ cheratinociti
❸ cellule endoteliali

Acido *cis*-Parinarico + Vitamina E
(perossidazione di membrana)

2',7'-diclorodiidro fluoresceina diacetato + GSH
(stato antiossidante intracellulare)

DPH (1,6-difenil-1,3,5-esatriene) Anisotropia
(fluidità di membrana)

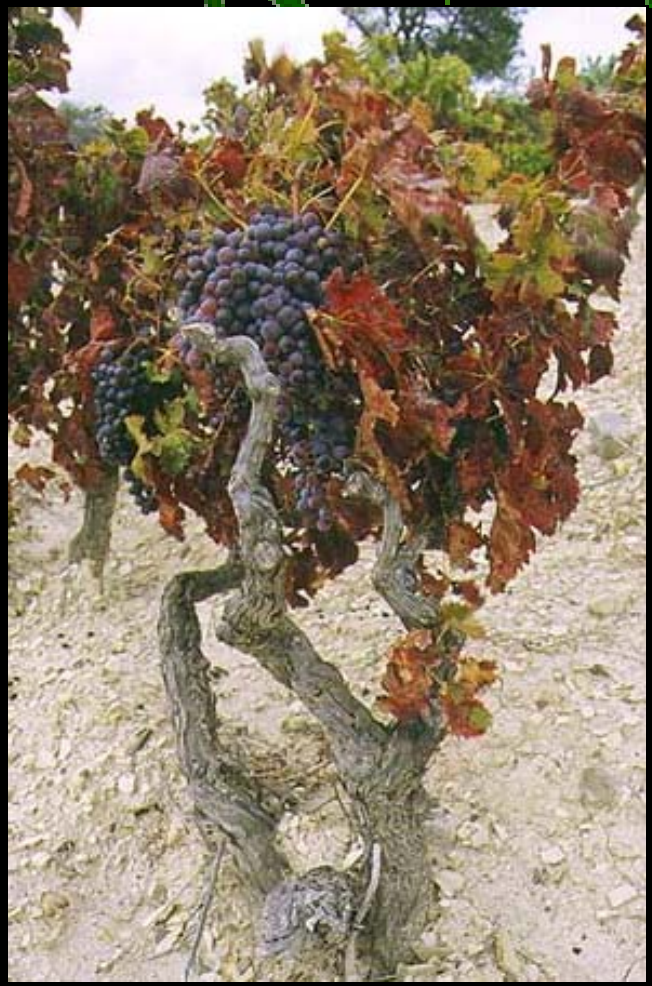
BODIPY-FL casein (*rilascio proteasi→ danno precoce)*

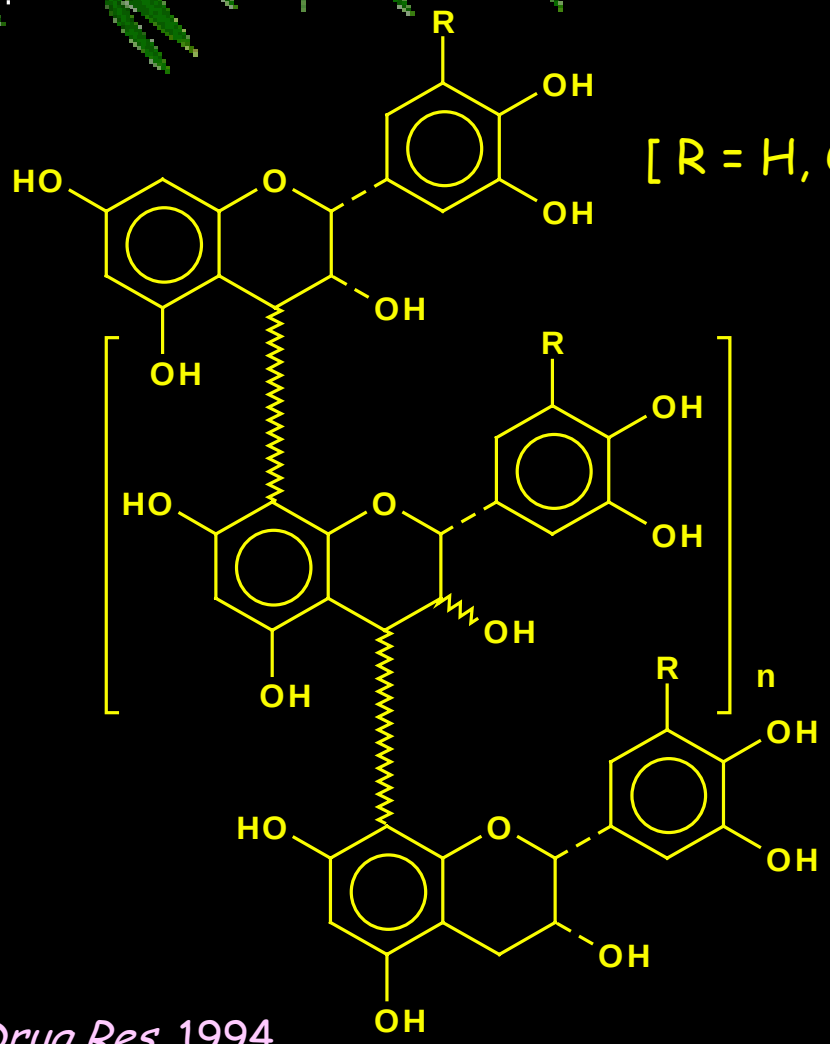
18th IFSCC Venice 1994

Free radical scavenging action and anti-enzyme activity of proanthocyanidin A2, a new poly-phenol from *Aesculus hippocastanum*.

R. Maffei Facino et al. Cosmetics & Toil. Ed. Am. 1996

Oligomeric procyanidins from *Vitis vinifera* seeds





[R = H, OH]

R. Maffei Facino et al. *Arzneim. Forsch./Drug Res.* 1994

Attività multicentrica → sostanze polifunzionali

i più potenti agenti antilipoperossidanti di origine naturale……

IC_{50} 0.05 μM Vitamina E 1.25 μM

…. con proprietà di rigenerazione vitamina E
e di stabilizzazione acido ascorbico….

…… ad elevata attività anti-enzimatica……

β-glucuronidasi	IC_{50} 1.1 μM
Xantina ossidasi	IC_{50} 2.4 μM
Elastasi	IC_{50} 4.2 μM
Jaluronidasi	IC_{50} 8.0 μM

….. potenti agenti ferro-chelanti (log K = 9.35)

Arzneim. Forsch./Drug Res. 1994; *Biofactors* 1997; *Planta Med.* 1998 + 2001;
Acta Phytother. 1998; *Life Sci.* 1999 + 2000 + 2003

19th IFSCC Sydney 1996

Protective effect of procyanidins from *Vitis vinifera* seeds on UV-induced photodamage : in vitro and in vivo studies.

Int. J. Cosmet. Sci. 1998

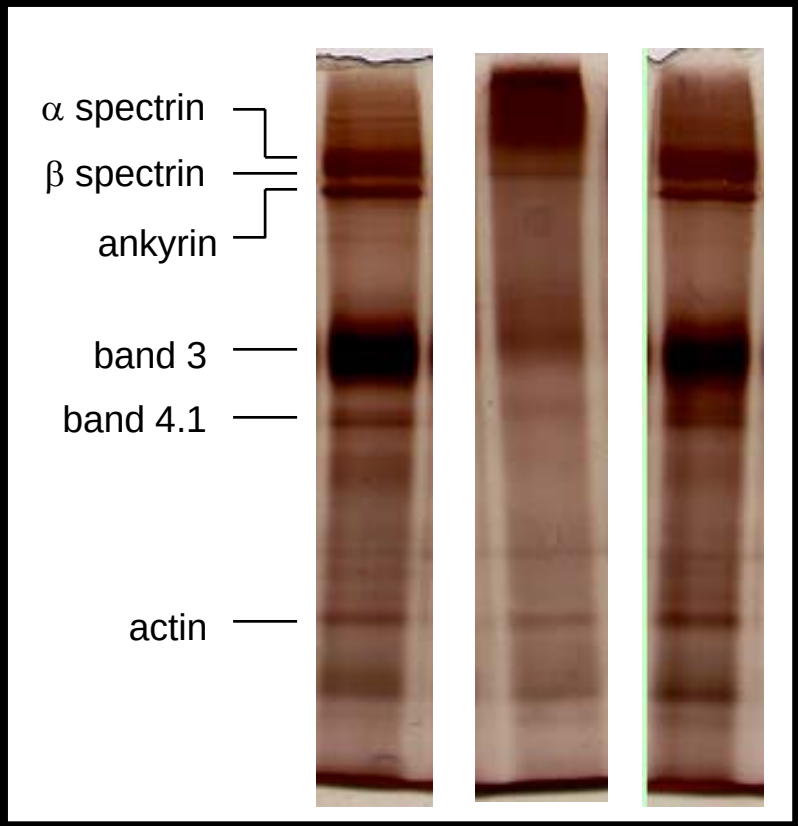
18th International Conference on Polyphenols Bordeaux 1996

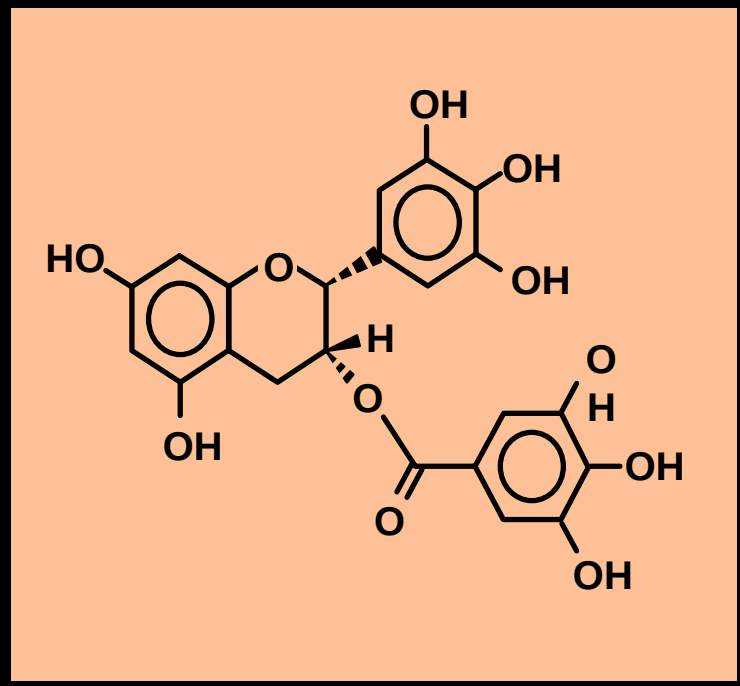
Antioxidant activity of green tea and regenerating ability on vitamin E : *in vitro* and *in vivo* studies

EGCG regenerates α-tocopherol via reduction of its phenoxyl radical (EPR experiments)

Biochem. Biophys. Res.Commun. 2003

UVB-induced damage to RBC membrane proteins: protective effect of green tea (SDS-PAGE)



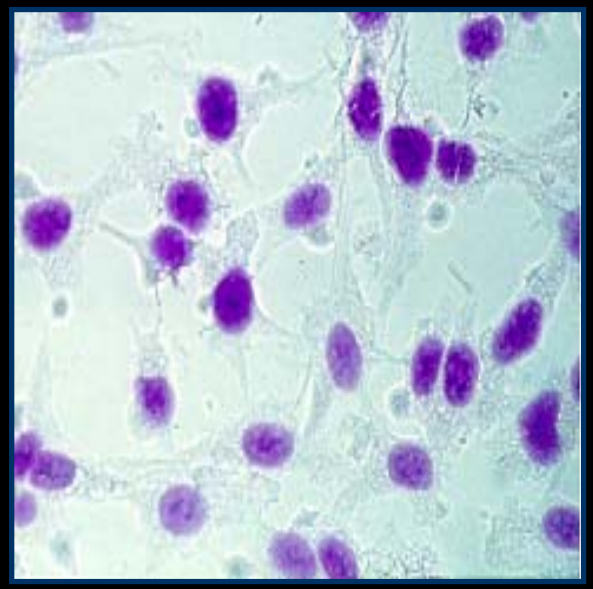


20th IFSCC Cannes 1998

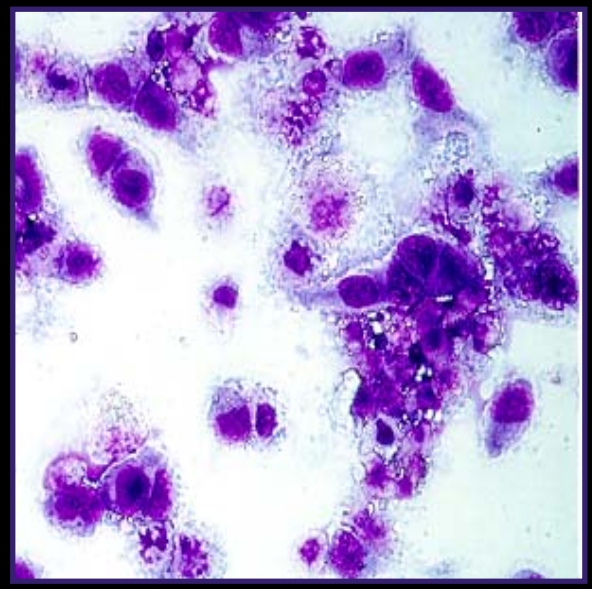
New bioanalytical markers of the oxidative stress status of human keratinocytes following UVB exposure

Il Farmaco 2000

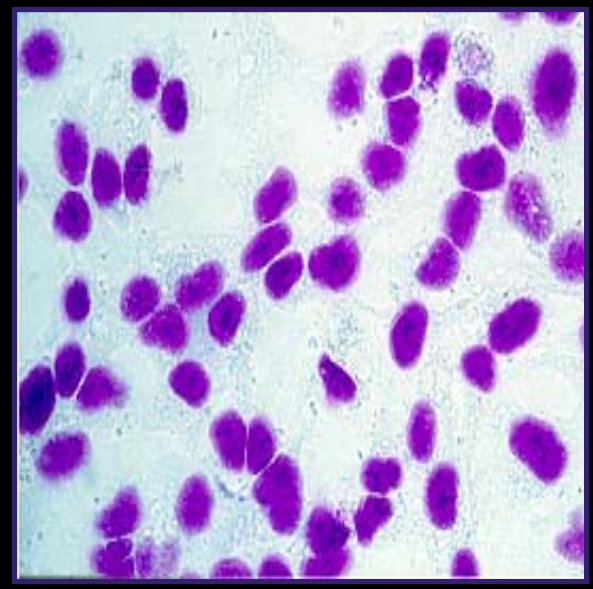
Cheratinociti: danno ossidativo indotto da UVB ed effetto protettivo dell'estratto di *Ratania*



Cellule controllo



Cellule irradiate



Ratania 20 μg/ml

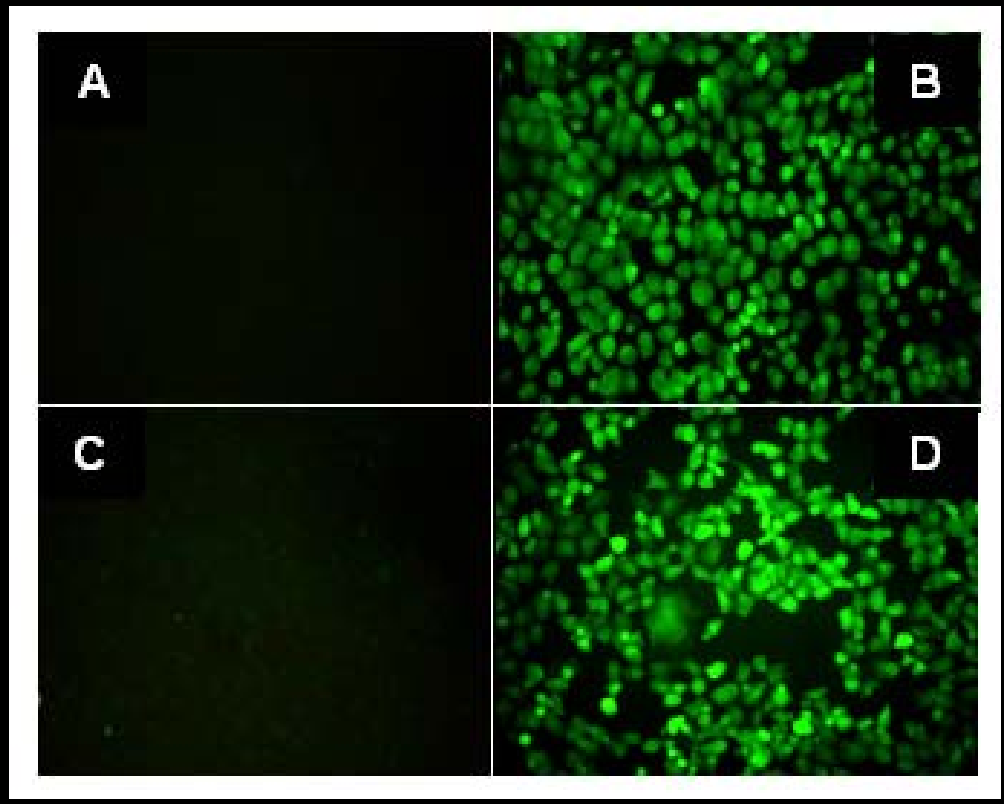
50 mJ/cm² - 24 h post irradiazione

Antioxidant and photoprotective activity of a lipophilic extract containing neolignans from *Krameria triandra* roots. *Planta Med.* 2002

Fluorescence microscopic analysis of intracellular DCF formation in keratinocytes: CuOOH as inducer

Controls

CuOOH



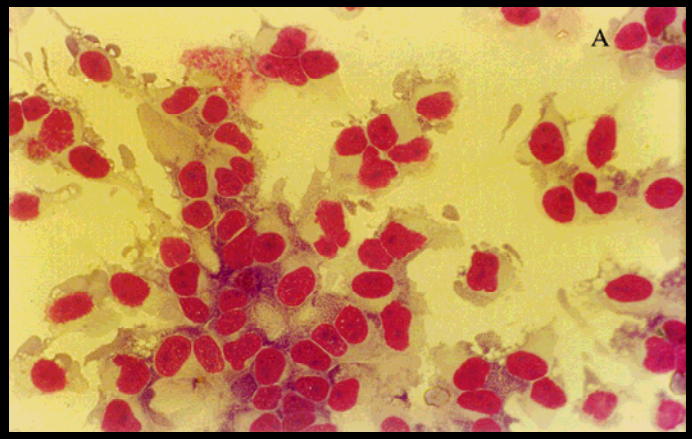
CuOOH + Green tea

CuOOH + GSH

Protective effect at intracellular level

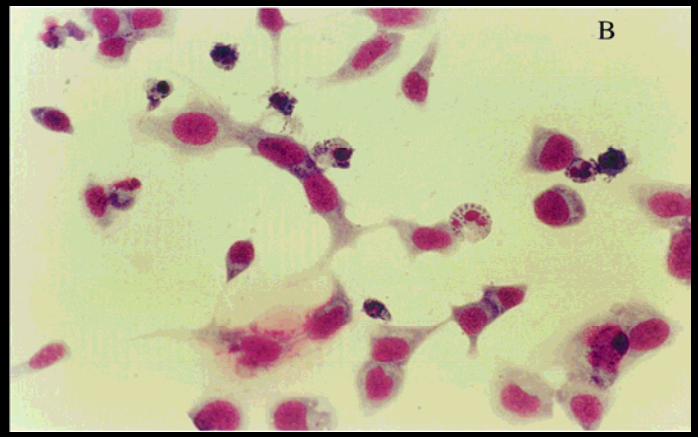
No protective effect

Keratinocytes: cell morphology (light microscopy) and TUNEL-positive cell counting



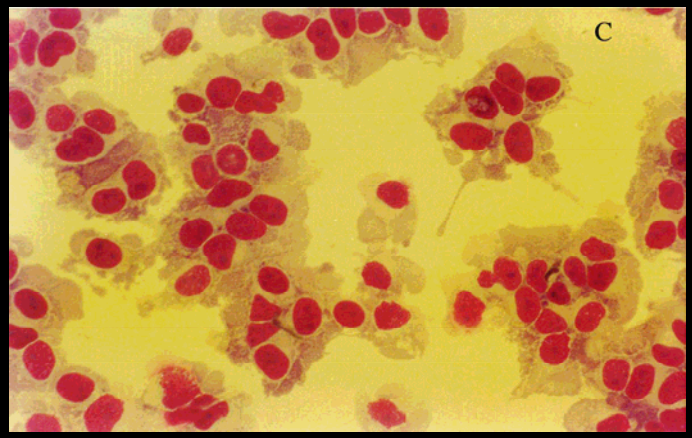
Control

2 ± 1 % apoptotic cells



UVB 50 mJ/cm^2
24 h post-radiation

22 ± 1.8 % apoptotic cells



UVB + NAC
no apoptotic cells

TUNEL (TdT - mediated dUTP nick end labelling) analysis

Il Farmaco 2000

XXI International Conference on Polyphenols Marrakech 2002

Antioxidant activity of polyphenols from solid olive residue of C.V. Coratina

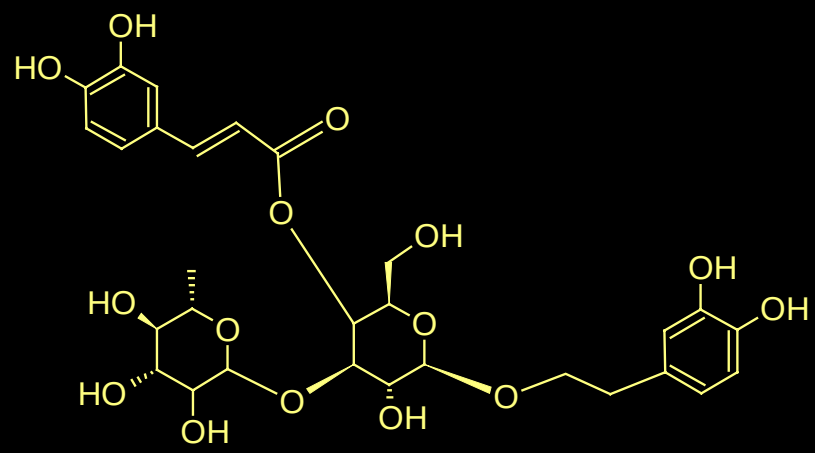
Aldini et al., *Fitoterapia* 2006

THAWED SOLID OLIVE RESIDUES

ENRICHED STANDARDIZED EXTRACT
TOTAL POLYPHENOLS: 16.0%

cell-free and cell-based experimental models

a by-product of olive oil extraction process is an industrial source of antioxidants



VERBASCOSIDE 58.8%

2000 →

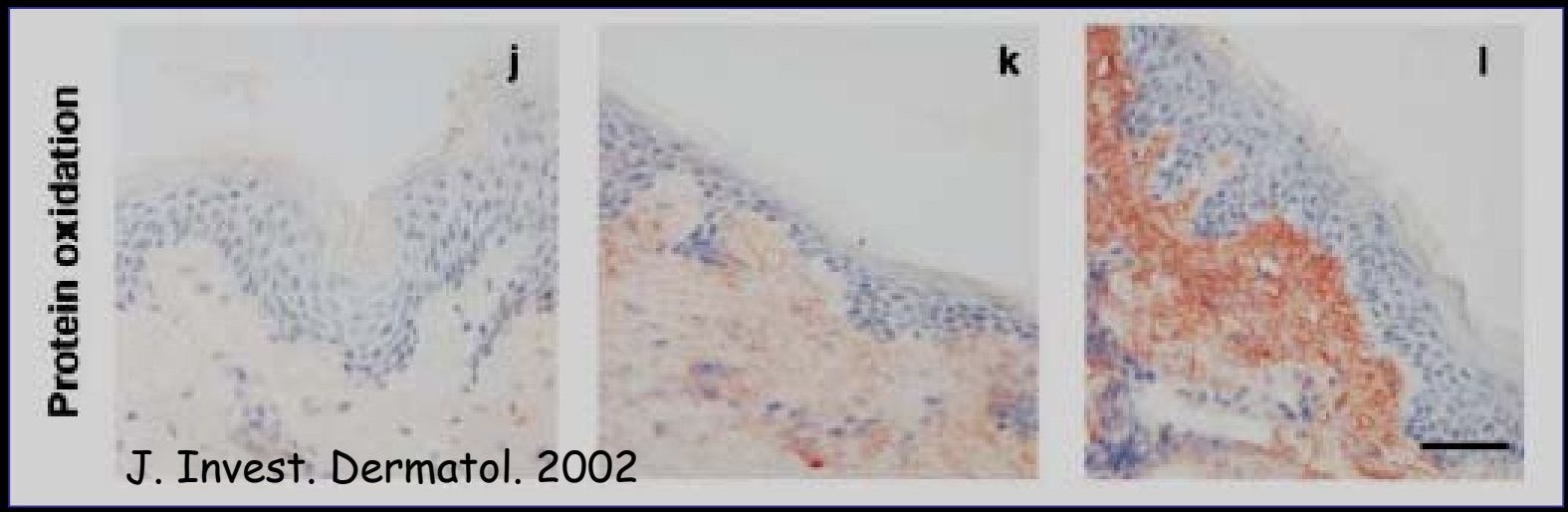
NUOVI APPROCCI MOLECOLARI NELLA PREVENZIONE DELL'INVECCHIAMENTO CUTANEO

↓

L'ipotesi dello stress carbonilico

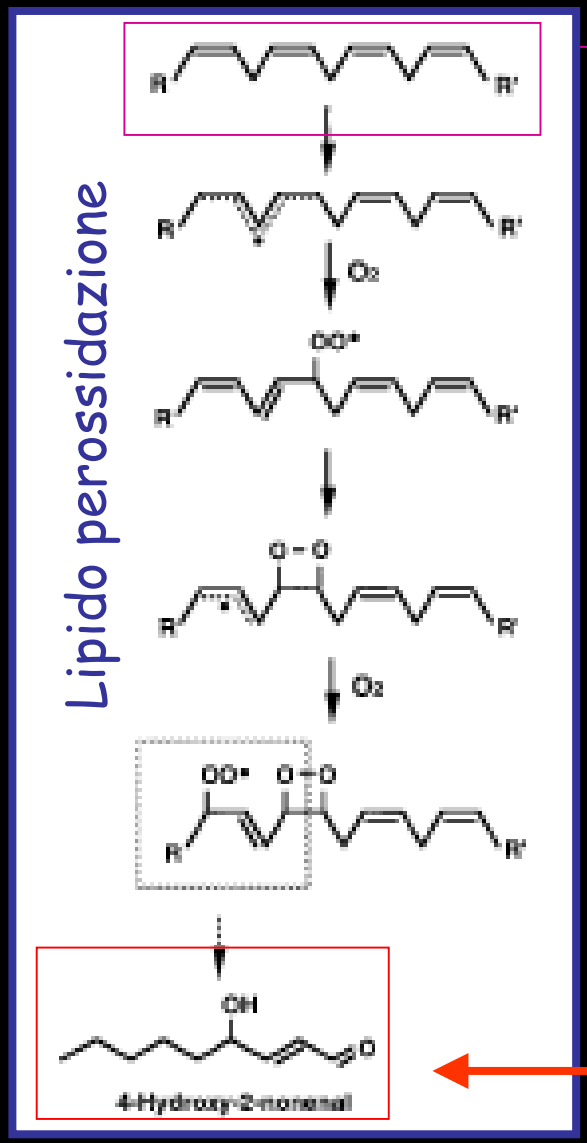
Immunohistochemical localization of carbonyl adducts in human skin

Young control skin | Aged skin | Photoaged skin



HNE protein adducts in actinic elastosis of photodamaged skin

4-idrossi-nonenale (HNE) come prodotto di perossidazione lipidica negli epiteli umani stratificati



PUFAs ω-6
(acido arachidonico, acido linoleico)

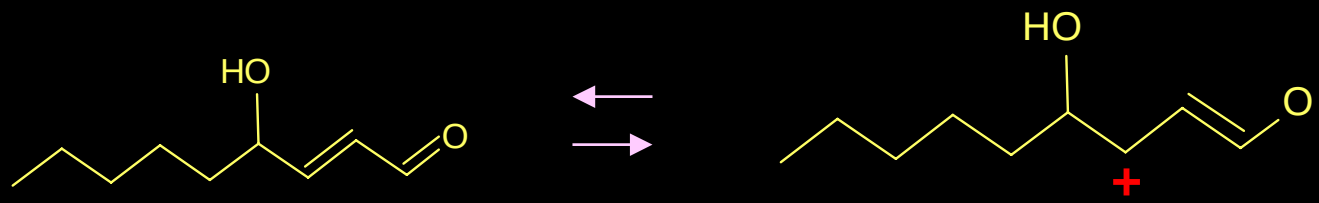
	Cheratinociti epidermide	Cellule mucosa orale
Acido linoleico	27.4 ± 0.5	14.5 ± 0.7
Acido arachidonico	6.3 ± 0.3	9.3 ± 0.4

Valori riportati come % degli acidi grassi totali

Terashi et al, *J. Dermatol. Sci.* 1999

Uchida et al. *J. Biol. Chem.* 1999

La reattività dell'HNE è dovuta alla natura elettrofila del C3

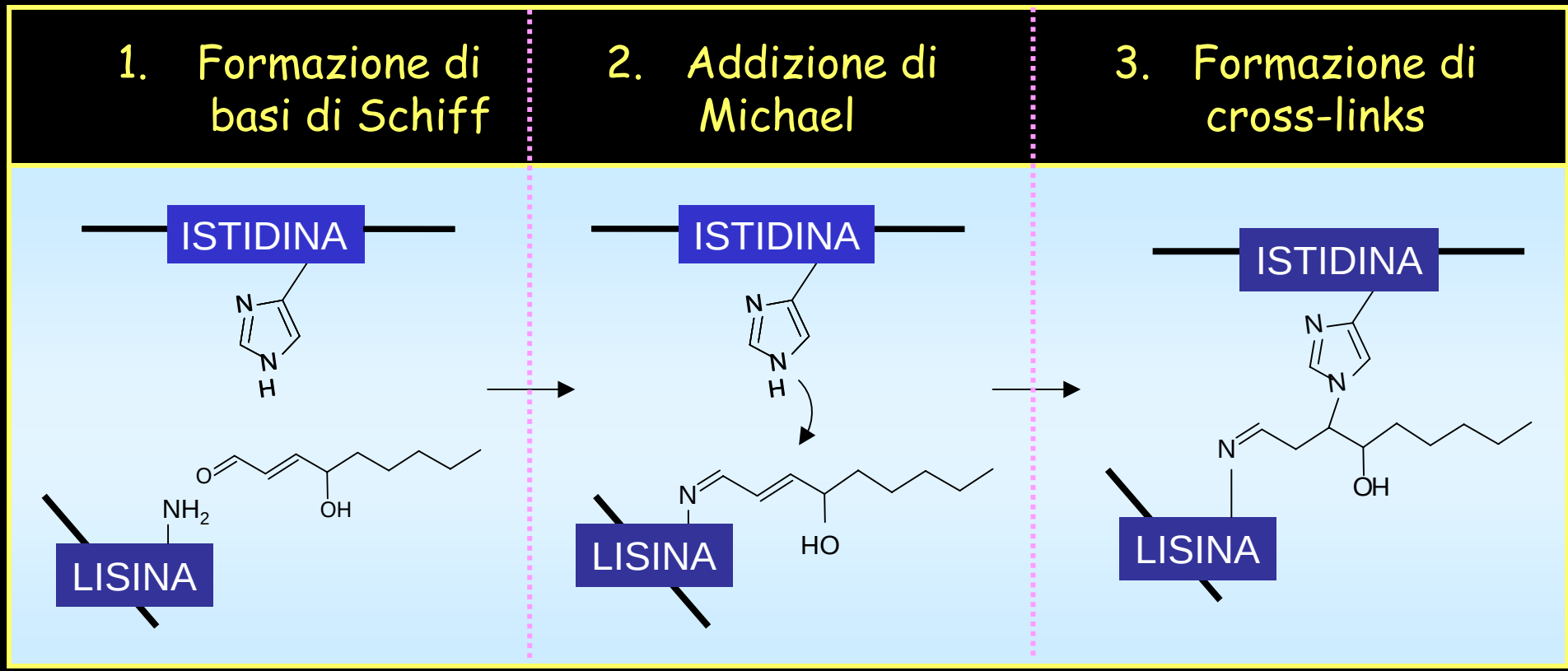


Legame covalente a siti nucleofili di

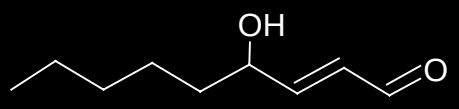
- Peptidi e Proteine (Cys, Lys, His)
- Aminofosfolipidi di membrana (PE)
- Basi nucleotidiche del DNA

MODIFICAZIONI STRUTTURALI IRREVERSIBILI → CITOTOSSICITA'

Aldeidi insature : meccanismo di formazione di ponti intra- e intermolecolari (crosslinks) a livello proteico

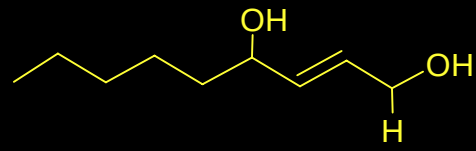


Metabolismo HNE
(epatociti, tessuto epatico, cardiaco)



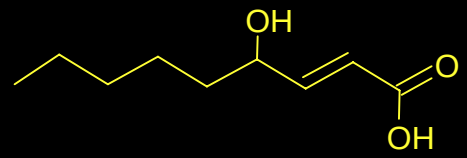
HNE

Aldoso reduttasi



1,4-diidrossinonene (DHN)

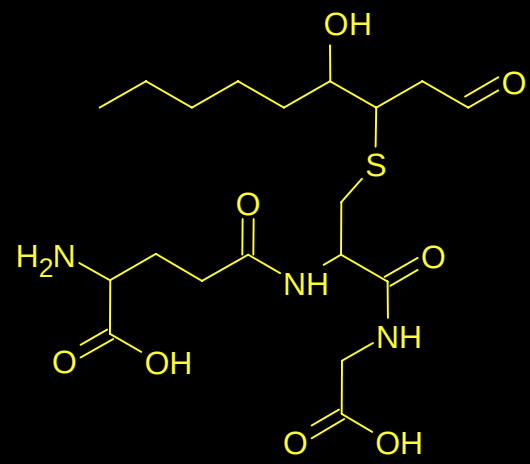
Aldeide deidrogenasi



Acido 4-idrossinonenoico (HNA)

Glutatione transferasi

GSH

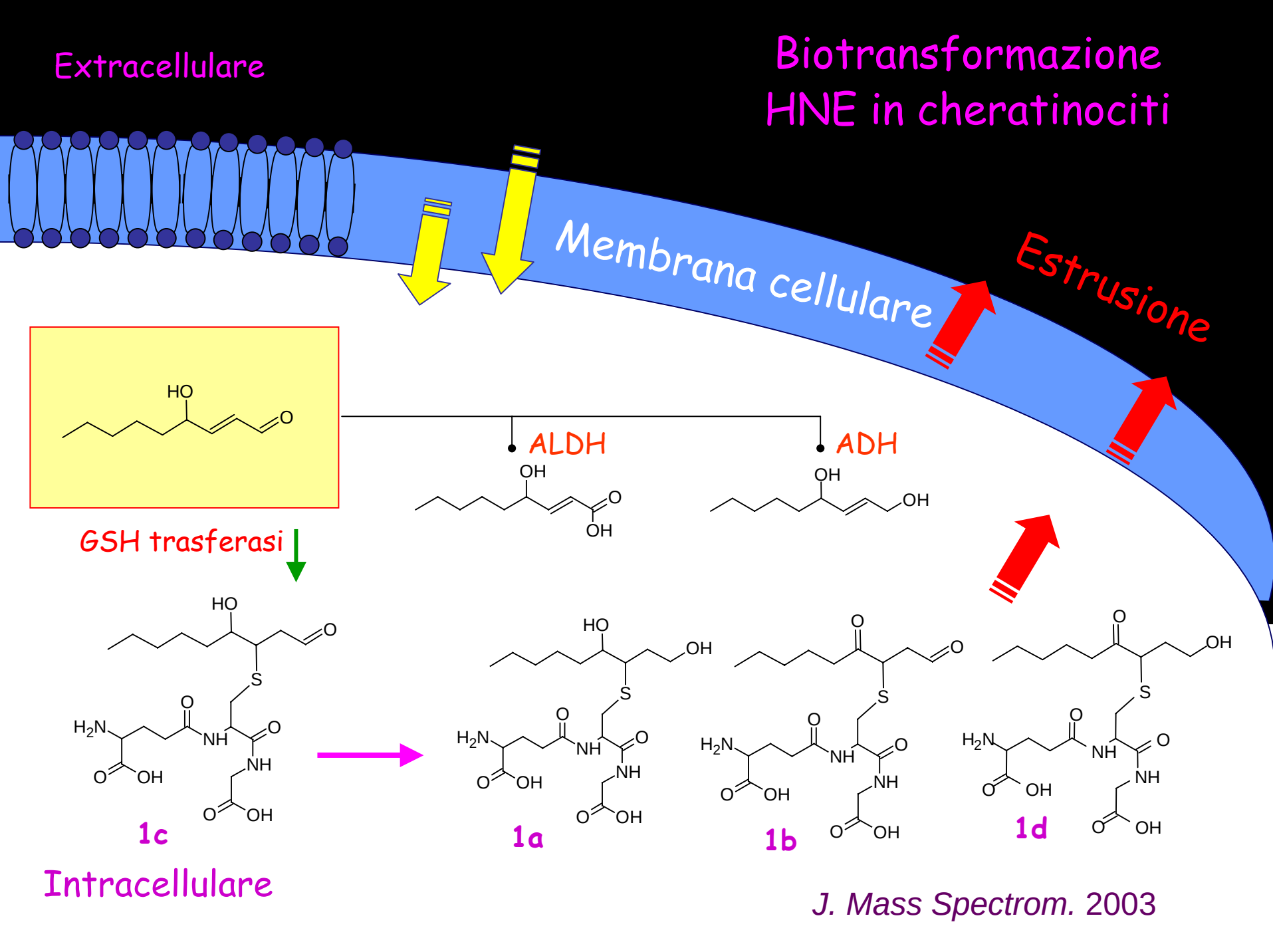


Addotti al GSH

Cheratinociti ??????

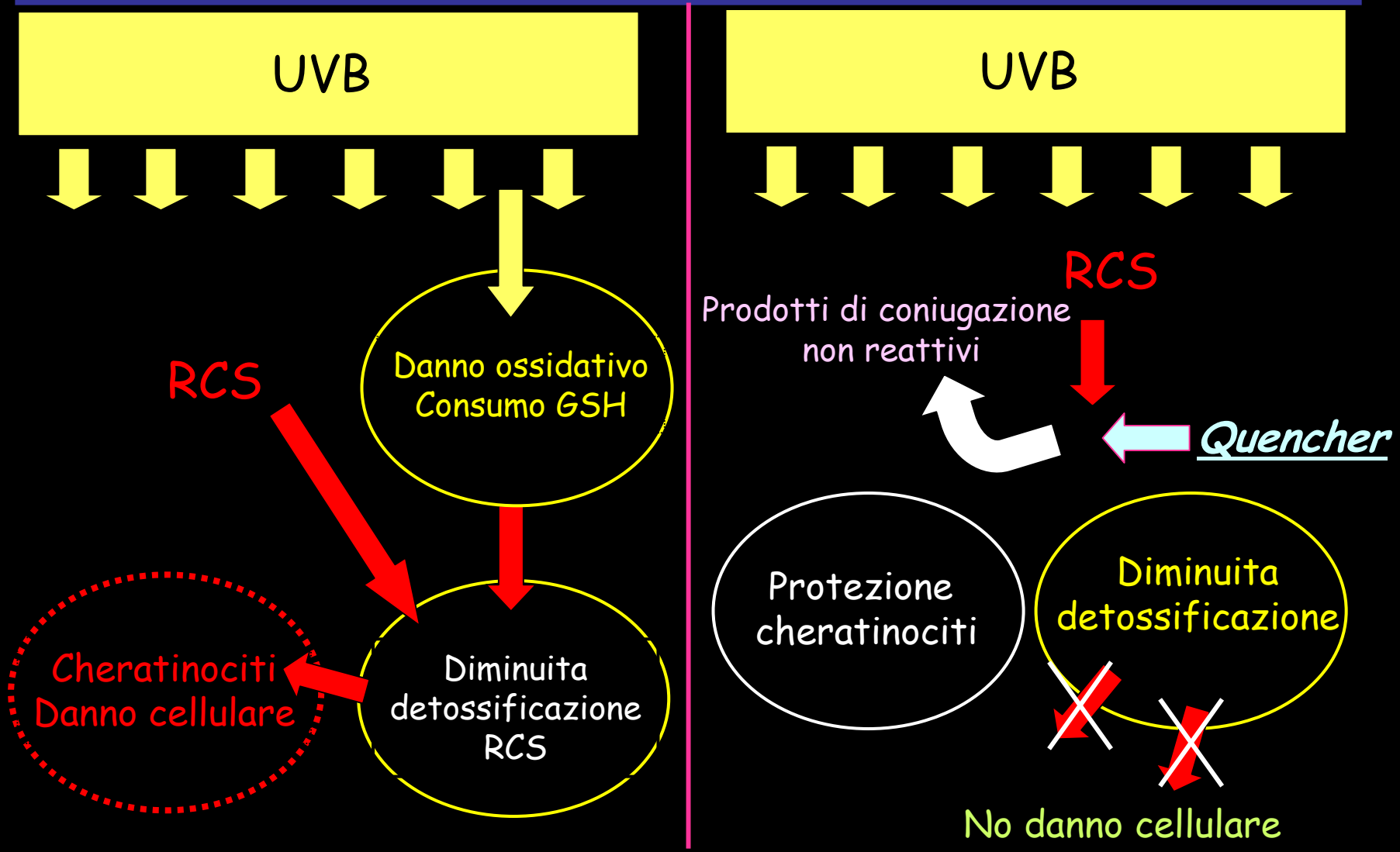
22th IFSCC Edinburgh 2002

Detoxification of cytotoxic unsaturated aldehydes in keratinocytes is a GSH-dependent pathway: inhibition by UVB and protective effect of histidine and cysteine-containing peptides



J. Mass Spectrom. 2003

Quencher di aldeidi (RCS): un nuovo razionale per la fotoprotezione ?



Identification of endogenous peptides as α,β-unsaturated aldehydes quenchers: a peptidomic approach

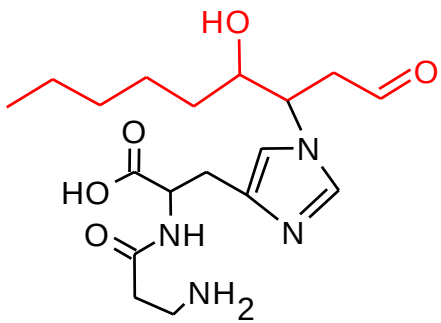
Biological matrix
(tissue homogenate)

HNE spiked

Incubation (37°C, 1h)

Peptides pool isolation (Ultrafiltration - *cut-off* 10 kDa)

Identification and characterization of peptides/HNE adducts
LC-ESI-MS/MS



Biochem. Biophys. Res. Commun. 2002
J. Mass Spectrom. 2002 + 2003
J. Chromatogr. B 2005

CARNOSINE as a prototype compound

IFSCC Conference – Firenze 2005

Tyrosyl-Histidine dipeptide:
a new approach against premature aging

DOVE STIAMO ANDANDO ?

❶ Prodotti naturali → Nuovi principi attivi
Nuove funzionalità
Stabilità metabolica

❷ Proteomica: caratterizzazione di proteine
e proteine modificate

❸ Sviluppo di inibitori di processi degenerativi
da stress carbonilico → peptidi e derivati

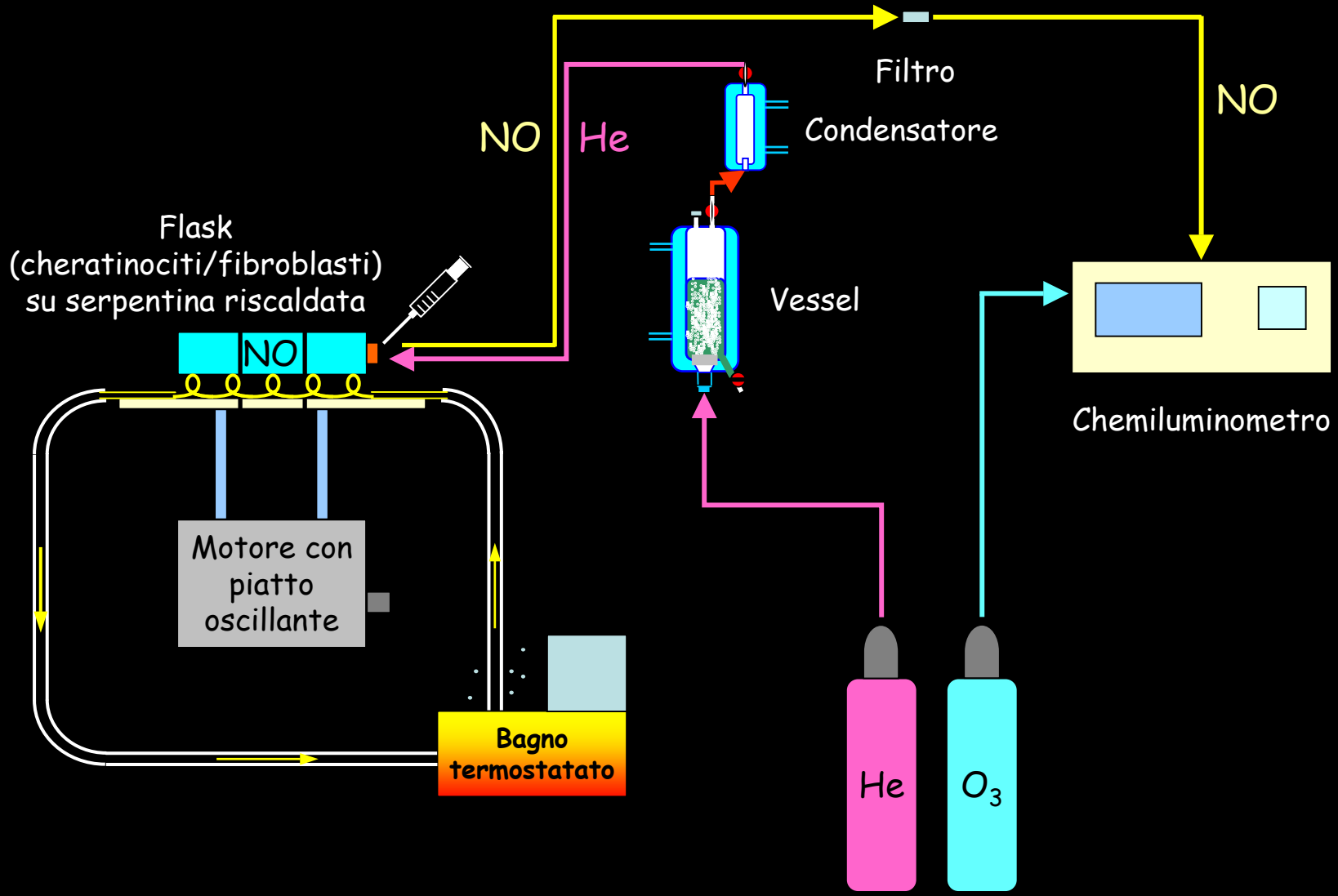
1 Prodotti di origine naturale

estratti, frazioni, principi attivi
quali modulatori della sintesi NO

NO → a powerful signaling molecule
in human skin involved in

- ⇨ skin homeostasis
- ⇨ vasodilation
- ⇨ inflammation
- ⇨ melanogenesis
- ⇨ immune response

Schema del sistema chemiluminometrico per l'analisi di NO rilasciato da colture cellulari

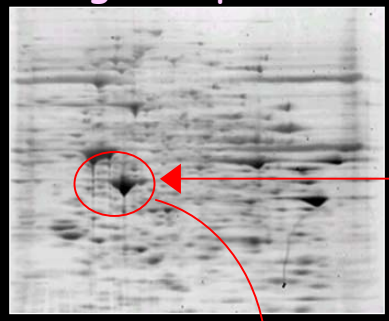


2 Identification of carbonylated proteins by a proteomic approach

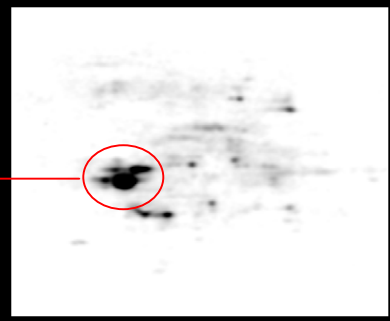
Biological matrices (cells, tissues, fluids)

Sample preparation

2D gel map



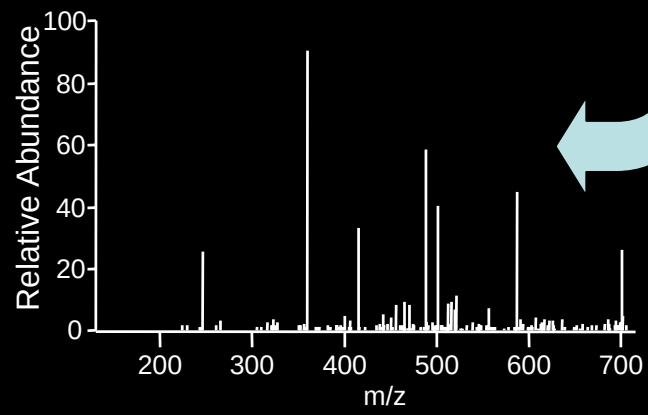
2D BLOT



Spot excision

In gel trypsin digestion

Mass spectrometry



Protein identification

MS strategies for protein carbonyls characterization

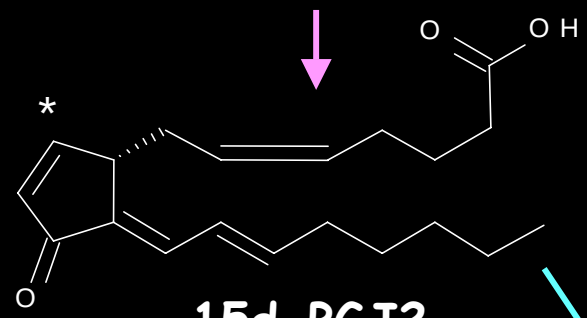
MS and MS/MS studies

- Identification of the aa site
- Characterization of the modification
- Elucidation of the adduction mechanism

Aldini et al., *J. Mass Spectrom*. 2006

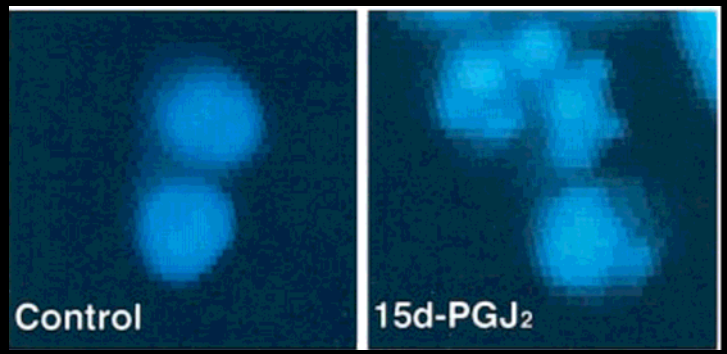
Identification of cell target of 15-deoxy-Δ12,14- prostaglandin J2 (15d-PGJ_2) by proteomic analysis

ARACHIDONIC ACID → PGD2



15d-PGJ2

APOPTOSIS



ACTIN as the main 15d-PGJ_2 protein target

↓

conformational change

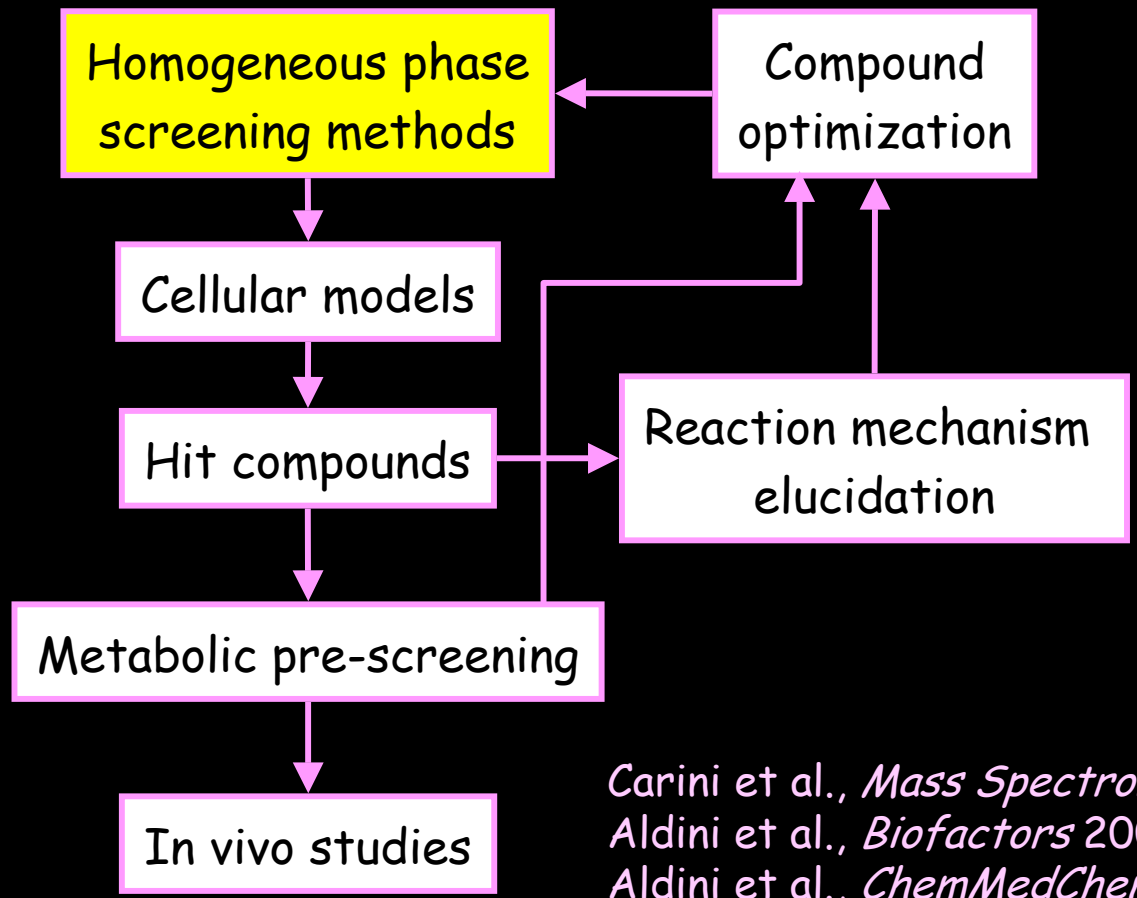
↓

disruption of the actin cytoskeleton

Aldini et al., *Biochemistry* (in press)

3

Discovery of RCS quenching compounds



Carini et al., *Mass Spectrom Rev* 2004
Aldini et al., *Biofactors* 2005
Aldini et al., *ChemMedChem* 2006
Aldini et al., *Med Res Rev* 2006
Dalle-Donne et al. *J Cell Mol Med* 2006

Sviluppo e applicazione di un metodo MS/MS per lo studio di reattività e specificità di potenziali *RCS sequestering agents*

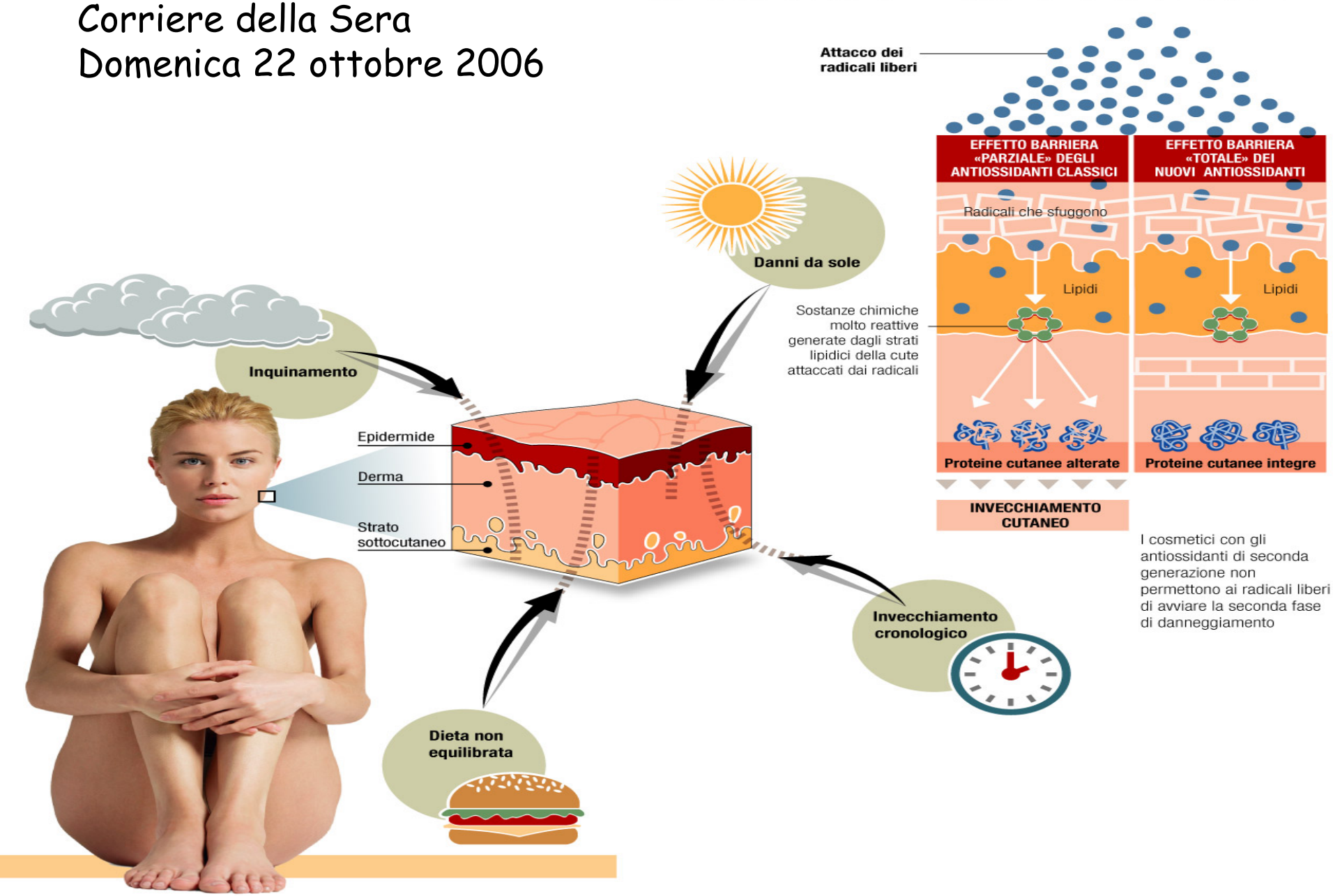
attività quenching dei derivati della carnosina (HNE)

Derivati Carnosina	UC*	Derivati Carnosina	UC
FL 926-A-003	0.744	FL 926-A-005	1.167
FL 926-L-003	0.949	CS-17	1.339
FL 926-A-009	0.96	FL 926-A-008	1.535
CARNOSINA	1.00	FL 926-A-006	1.667
FL 926-A-002	1.008	FL 926-A-001	1.751
FL 926-A-004	1.131	FL 926-A-010	1.965

*I valori sono espressi come "unità di carnosina"

Corriere della Sera
Domenica 22 ottobre 2006

Come contrastare l'invecchiamento della pelle



I cosmetici con gli antiossidanti di seconda generazione non permettono ai radicali liberi di avviare la seconda fase di danneggiamento

Gli antiossidanti cercano di bloccare l'azione dei radicali liberi, dannosi per la nostra pelle. I radicali derivano dall'azione combinata di più fattori, alcuni «esterni»: sole e inquinamento, e alcuni «interni»: l'inevitabile invecchiamento cronologico, accelerato da una dieta povera di frutta e verdura

Istituto Chimica Farmaceutica e Tossicologica P. Pratesi

- Valutazione in vitro delle proprietà di assorbimento/ penetrazione di sostanze cosmetiche attraverso cute di origine umana (Guideline COLIPA)
- Forme di dosaggio cosmetiche a rilascio modificato (cerotti e microsfere)

UNIVERSITÀ DEGLI STUDI DI MILANO

Istituto di Chimica Farmaceutica e Tossicologica P. Pratesi

CORSO DI PERFEZIONAMENTO (POST LAUREA)

SCIENZE COSMETICHE

Direttore: M. Carini

UNIMI research group

Bioanalysis and MS unit

R. Maffei Facino
G. Aldini
M. Orioli
G. Beretta
L. Gamberoni

Molecular Modelling

G. Vistoli
A. Pedretti

Biochemistry

A. Milzani
I. Dalle-Donne

RCS synthesis

E. Santaniello

Carnosine and derivatives

Flamma (Negrisoli and Canevotti)

Collaborations

- K. Uchida, Nagoya University, Japan
- B. Testa, University of Lausanne, Switzerland
- Cusi, Policlinico Multimedica, Milano
- KJ Yeum, Tuft's University HNRC, Boston

1980

Perlier

Hanorah

Variati

Indena

Curt Georgi – Universal Flavors

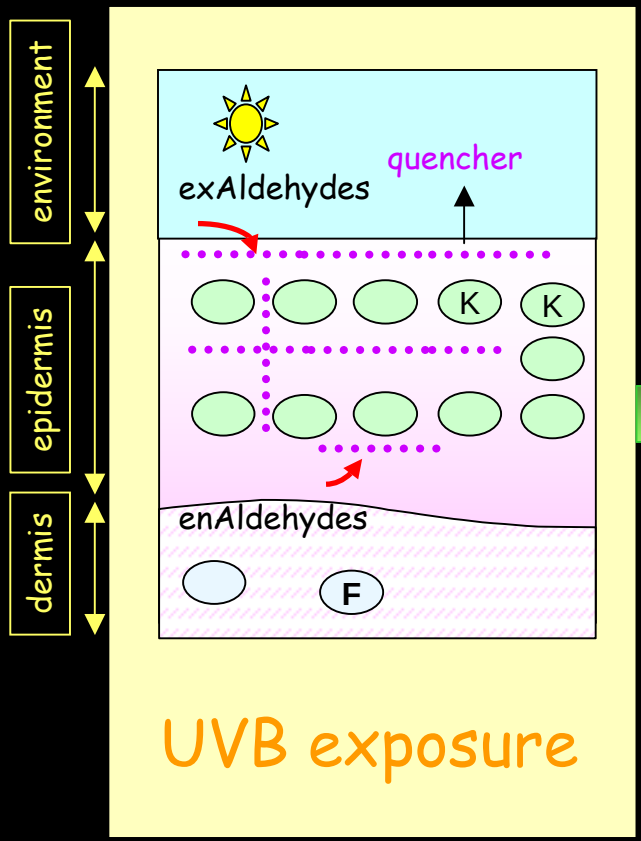
Pharmaton

Bottega verde

Flamma

Schiapparelli Pikenz

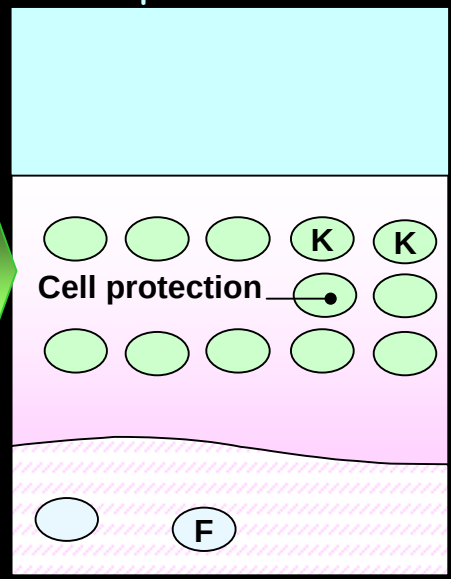
A new rationale for skin photo-protection



Quencher of cytotoxic reactive aldehydes

Reaction products: hydrophilic and unreactive adducts

Cellular and extracellular protection



K keratinocytes

F fibroblasts

Extracellular matrix

exAldehydes: exogenous cytotoxic aldehydes (acrolein)

enAldehydes: endogenous derived cytotoxic aldehydes (HNE, MDA)