



# RonaCare® Cyclopeptide-5

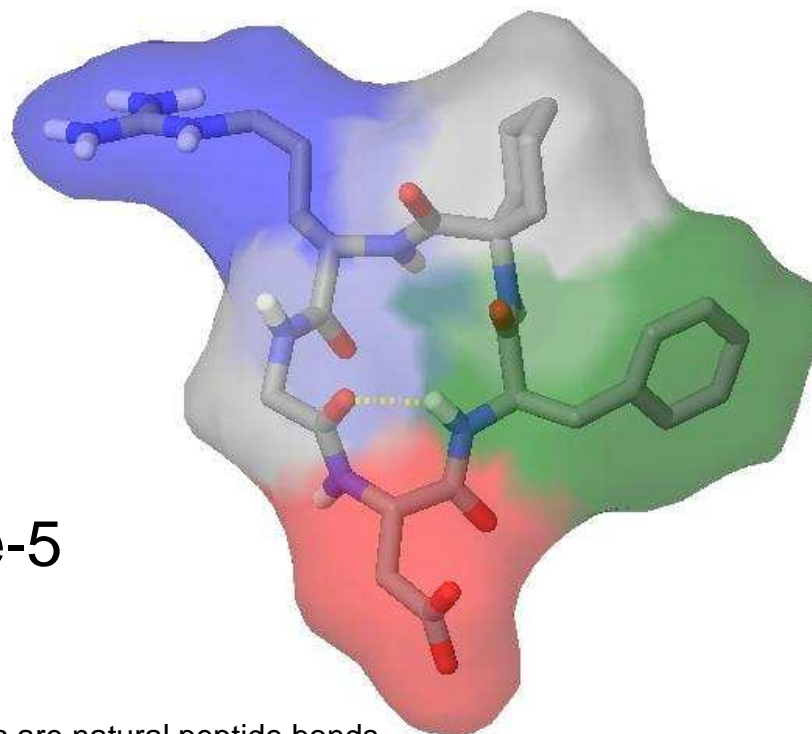
Ringling in a unique, new peptide generation

March 2010

# Smart molecule design



Merck researchers achieved a break-through in designing the **first cyclic** and homodetic\* peptide for **cosmetic** applications



RonaCare® Cyclopeptide-5

\* all of the covalent linkages between the amino acids are natural peptide bonds

# RonaCare® Cyclopeptide-5



RonaCare® Cyclopeptide-5 stands out from all commercially available peptides being used in cosmetic applications due to its unique structure as a cyclic shaped agent.

## Providing Anti-aging benefits

- Wrinkle reduction
- Firmness
- Elasticity

# The lock-and-key-model

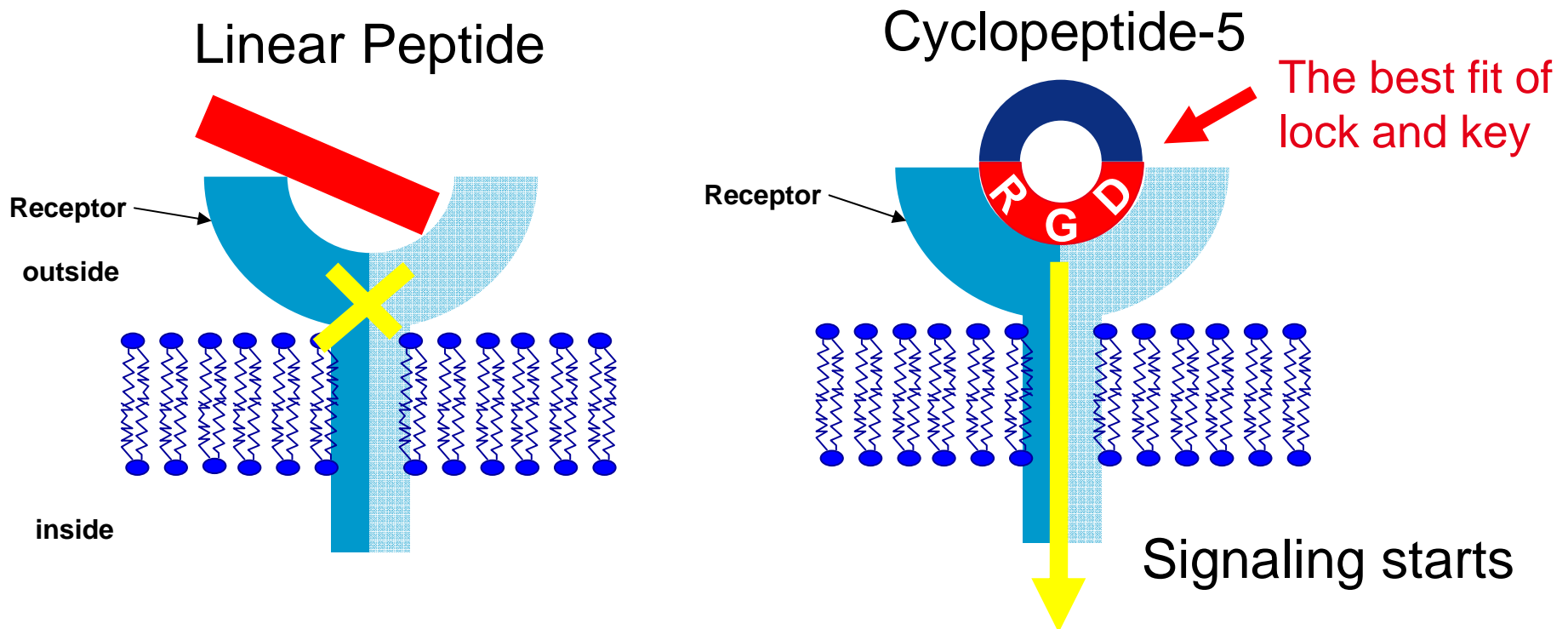
In essence, different peptides produce different biological effects by signalling cells to perform various functions by attaching to “receptors” found on the surface of each cell. You think of the **peptides as key** and the skin cell-surface **receptors as locks**.

**This is the lock-and-key-model.**

# What makes Cyclopeptide-5 different ?

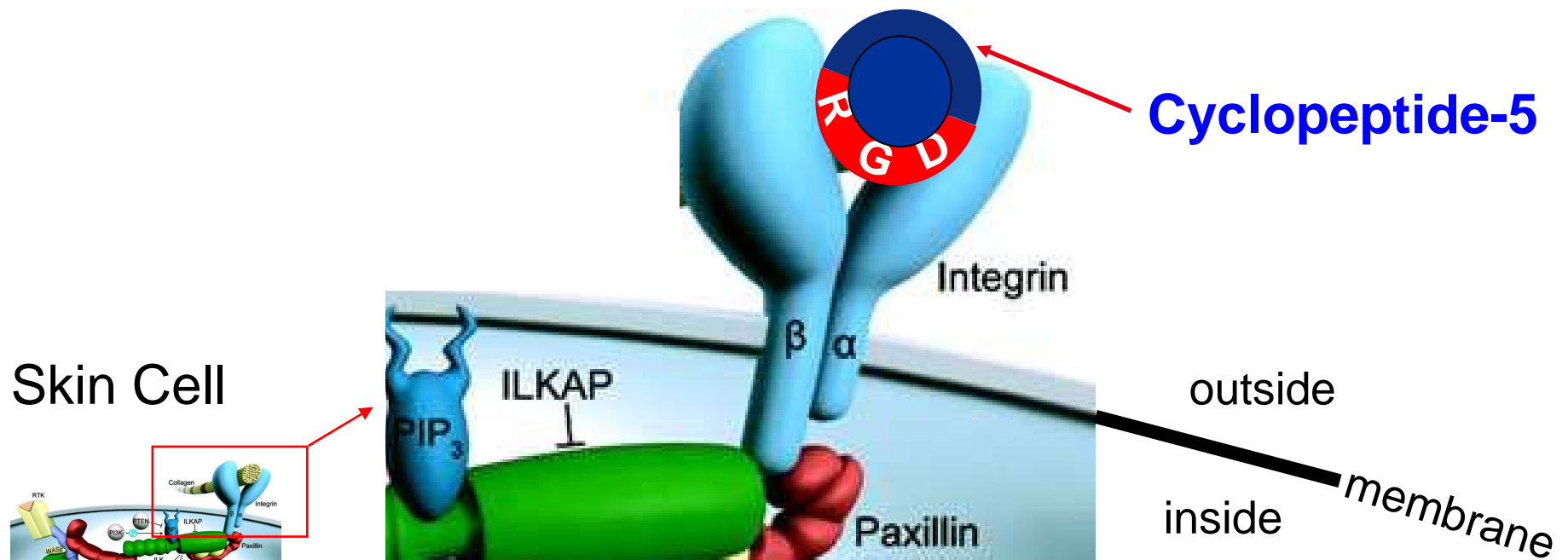


Its *cyclic* structure



# What is the biological target of Cyclopeptide-5 ?

This revolutionary peptide shows **high selectivity** to **certain receptors – like  $\alpha\beta 5$  and  $\alpha\beta 6$  integrins**



C. Brakebusch, R. Fässler (2003) The integrin-actin connection, an external love affair, *The EMBO Journal*, **22(10)**, 2324-2333.

# How Cyclopeptide-5 works



With such **integrins as lock** and **RonaCare® Cyclopeptide-5 as key**, it is shown that it is possible to mimic natural processes of skin communication and repair.

**Skin turns more elastic and firmer – and appears to have fewer wrinkles**

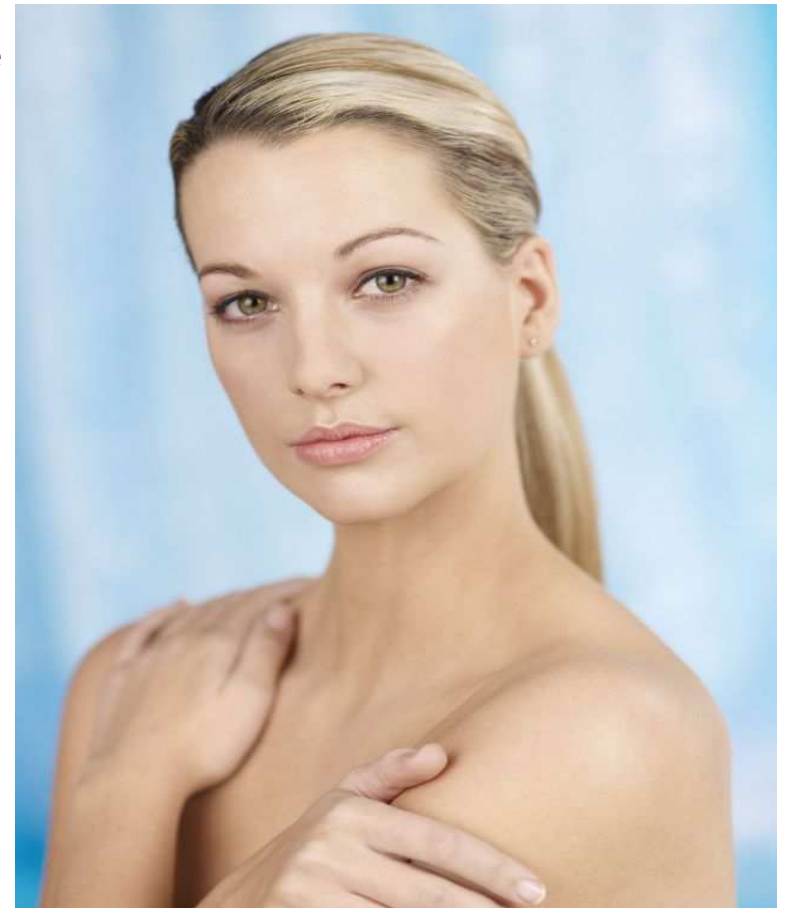


# RonaCare® Cyclopeptide-5

the first of a new peptide generation for cosmetics

Equipped with the outstanding edge of RonaCare® Cyclopeptide-5, you can create cosmetic products that work with the ultimate in precision.

**Welcome to a new dimension in effective anti-aging**





# RonaCare® Cyclopeptide-5

the first of a new peptide generation for cosmetics



**Find out more about this exciting peptide story.....**

# Smart molecule design



RonaCare® Cyclopeptide-5 was designed with **three smart features**

- A cyclic structure
- A cRGD motif
- Special amino acids increasing its activity, selectivity and stability

# Smart molecule design – the cyclic structure



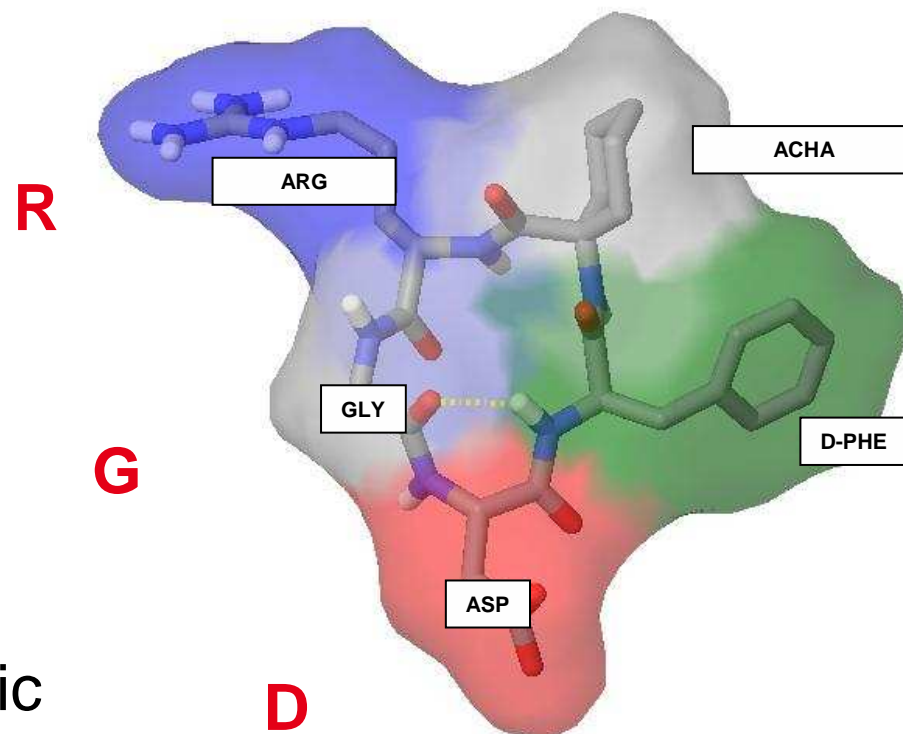
- The cyclic form of the peptide provides a high rigidity and makes it highly selective to the desired biological target – the integrin RGD binding site
- The cyclic conformation enhances its stability

# Smart molecule design - the cRGD motif



Cyclopeptide-5 consists of

- Arginine (R)
- Glycine (G)
- Aspartate (D)
- D-Phenylalanine
- Aminocyclohexane carboxylic acid (ACHA)



# Smart molecule design - the cRGD motif



The cRGD motif stands for the cyclic structure of the following amino acid sequence: [arginine-glycine-aspartate](#)

The cRGD motif is the best configuration to mimic natural processes at the chosen biological target site – [the integrins](#)

# Smart molecule design – special amino acids



Why did we use D-Phenylalanine ?

- D-amino acids stabilize the peptide against proteases
- additionally the aromatic D-amino acids adjust in that position of a cRGD peptide a defined conformation hereby **increasing** the ligand-receptor interaction (**activity and selectivity**)

# Smart molecule design – special amino acids



Why did we use Aminocyclohexane carboxylic acid (ACHA) ?

- ACHA is a symmetric non-natural amino acid (optically inactive) which achieves a high solubility
- With ACHA an optimum shape of the cRGD could be designed

# Cyclopeptide-5 – its biological target



What are Integrins ?

- Integrins are cell surface receptors  
(the lock in the lock-and-key-model)
- Integrins bind to cell surface and ECM components such as fibronectin, vitronectin, collagen and laminin  
(the keys in the lock-and-key-model).
- Cyclopeptide-5 takes over the role as a key



# Cyclopeptide-5 – its biological target



Integrins have two main functions:

- Attachment of the cell to the ECM
- Signal transduction from the ECM to the cell

# Cyclopeptide-5 – its biological target



## Attachment of the cell to the ECM

Integrins couple the ECM outside the cell to the cytoskeleton inside the cell

The connection between the cell and the ECM may help the cell to endure pulling forces without being ripped out of the ECM

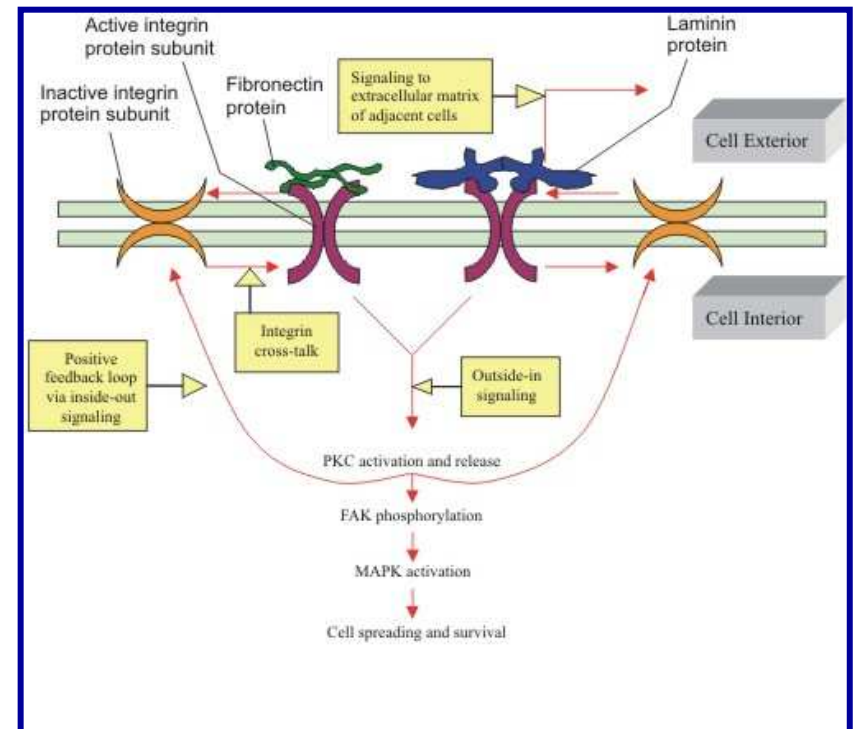
# Cyclopeptide-5 – its biological target



## Signal transduction

Integrins are responsible for transmitting signals between skin cells and the extracellular milieu

- by doing outside-in signalling
- and inside-out signalling



## Integrins and their signaling pathways

David A. Barron: Rice University

[http://www.ruf.rice.edu/~rur/issue1\\_files/barron.html](http://www.ruf.rice.edu/~rur/issue1_files/barron.html)

# Cyclopeptide-5 – its biological target



## Keratinocyte Integrins

- Keratinocytes express a number of integrins being essential for their anchorage and migration
- Some of them are constitutively expressed
- Some are expressed or up-regulated upon stimulation – like wound healing

# Cyclopeptide-5 – its biological target



RonaCare® Cyclopeptide-5 shows high selectivity respectively is a ligand for integrins – like  **$\alpha\nu\beta 5$  and  $\alpha\nu\beta 6$**

- **$\alpha\nu\beta 5$  is a constitutively expressed epidermal integrin – but also being expressed during the wound healing process**
- **$\alpha\nu\beta 6$  is an expressed epidermal integrin being induced during the wound healing process**

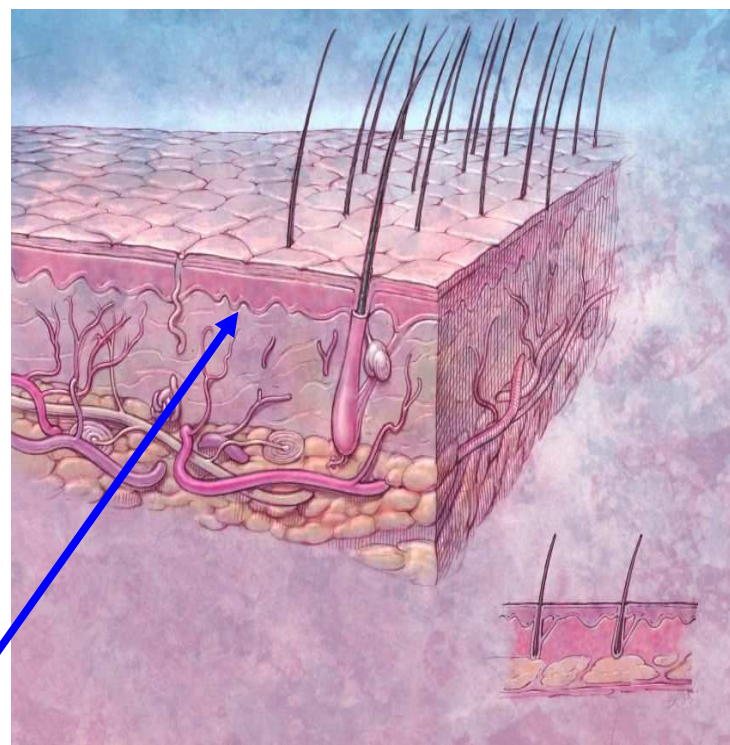
# Scenes of aging -

How Cyclopeptide-5 comes in here



Histological changes of the skin due to the ageing process can be found in

- The epidermis – thinning
- The dermis – reduced compacting
- **The basement membrane –  
loss in the distinctive wave structure**



# Scenes of aging



## The basement membrane

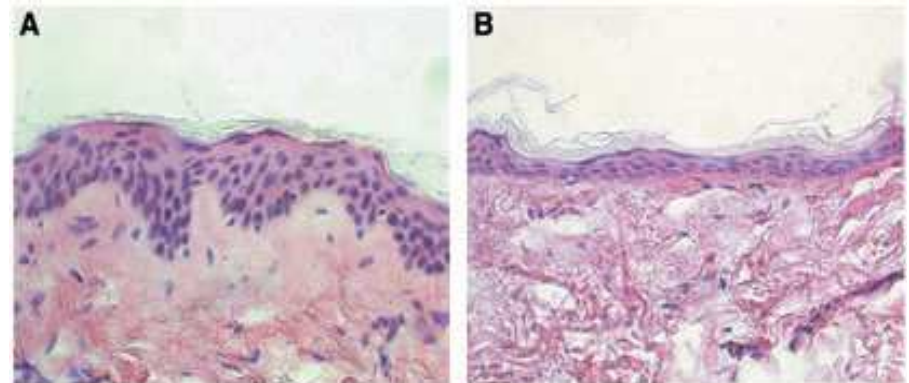
- is, together with other components, responsible for the connection between the dermis and epidermis, the so-called dermo-epidermal-junction (DEJ).

# Scenes of aging



## The aging of the basement membrane

This typical change in the DEJ is related to a reduction of protein expression and an increase in the destruction of supporting DEJ fibres. The main consequences are the flattening of the basement membrane and multiple functional und structural changes of its components



**Histological difference of young (A, 28 years old) and intrinsically aged skin (B, 69 years old).**  
O. Holtkötter (2005) Unveiling the molecular basis of intrinsic skin ageing,  
*International Journal of Cosmetic Science*, **27**, 263-269.



# Scenes of aging



Functional changes of the basement membrane can be

- Migration of keratinocytes
- Liberation of enzymes
- *Defective communication between dermis and epidermis*

# Scenes of aging



The changes of the basement membrane due to the aging process lead to....



**...less elasticity and firmness of the skin**

# RonaCare® Cyclopeptide-5



**A unique peptide of a new generation comes in here.....**

- mimicking natural processes of skin communication and repair
- reviving the communication process between dermis and epidermis
- stimulating the production of important proteins of the basement membrane
- protecting ECM-structures against enzymatic degradation

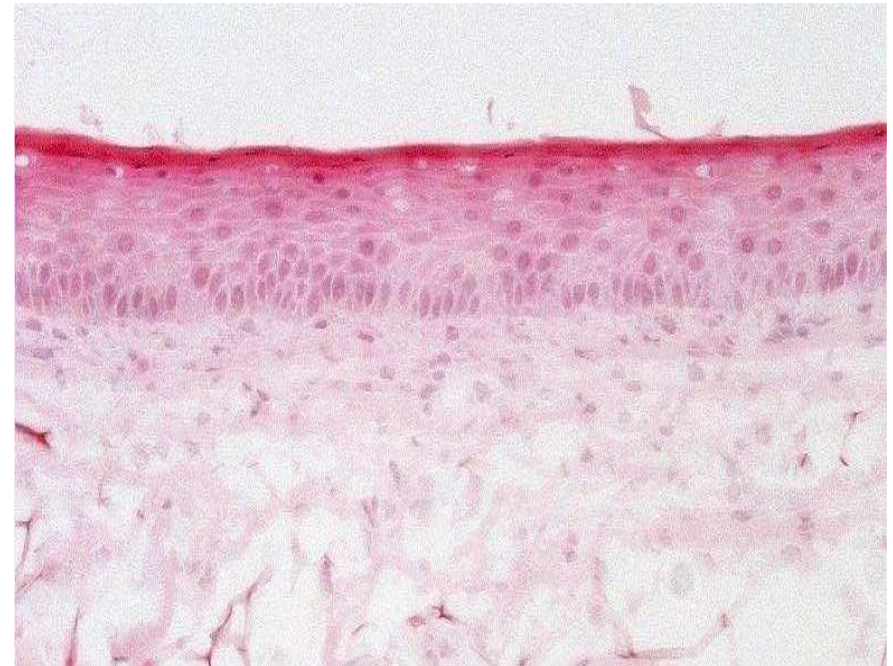
**.....with high selective activity**

# Anti-Aging efficacy *in vitro*:



## cDNA-Microarray study

- up-regulation of genes stimulating important basement membrane proteins and growth factors, e.g. laminin IV, collagen IV.
- A full thickness skin equivalent consisting of dermis and epidermis has been used as testing model with 0,3 ppm Cyclopeptide-5

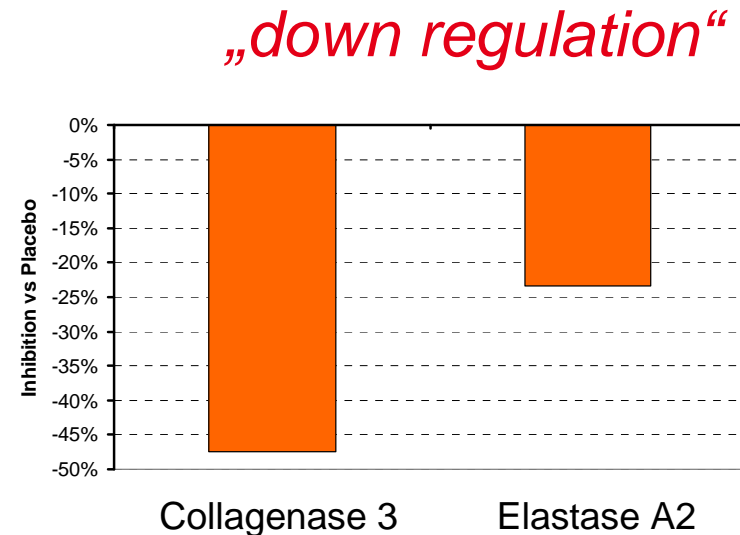


# Anti-Aging efficacy *in vitro*: Example



“down” of Collagenase 3: ~ - 47%  
“down” of Elastase A2: ~ - 23%

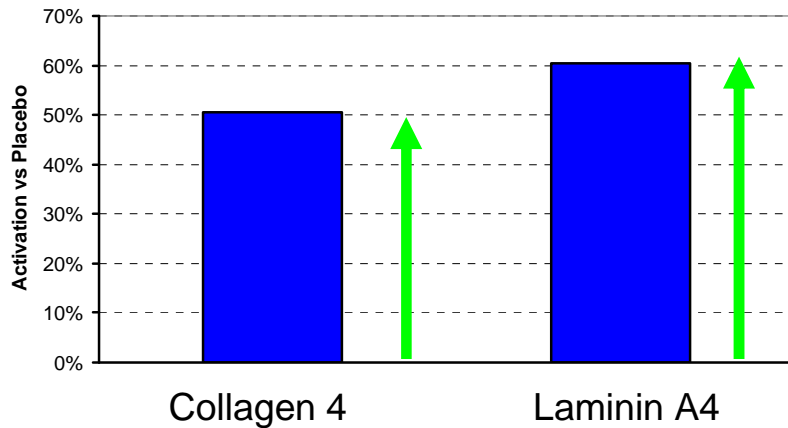
Cyclopeptide-5  
protects ECM-structures against  
enzymatic degradation



# Anti-Aging efficacy *in vitro*: Example



„up-regulation“



“up” of Collagen-4: ~ + 50%

“up” of Laminin A4: ~ + 60%

Cyclopeptide-5  
stimulates the production of  
important basement membrane  
proteins

# Anti-Aging efficacy *in vitro*:

## Laminin

- is a glycoprotein and part of the ECM
- is forming the basement membrane together with **Collagen type IV** and other components
- binds to cell surface receptors and thereby tightly connects the basement membrane to the adjacent cell layer. This ensures the stabilization of cellular structures.

# Anti-Aging efficacy *in vitro*:

## Collagen type IV

- is the netforming type of all collagen types
- it is found primarily in the basement membrane



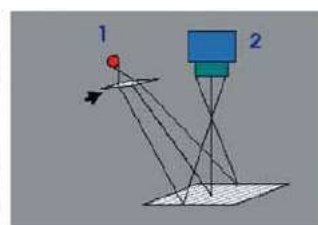
# Anti-Aging efficacy *in vivo*



## Design clinical study I (Crows` feet)

- Surface evaluation by means of Primos at day 0 and 28
- 20 volunteers (n = 20, f) with normal skin
- Female, Age range: 37-63 years (average: 44,8)
- Test area: Crows` feet
- application of test products twice daily for 28 days
- Testproducts (verum vs. untreated, 2 mg/cm<sup>2</sup>)

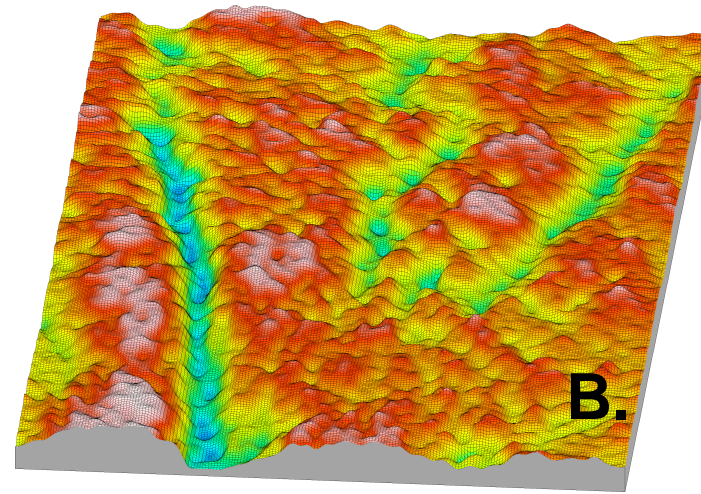
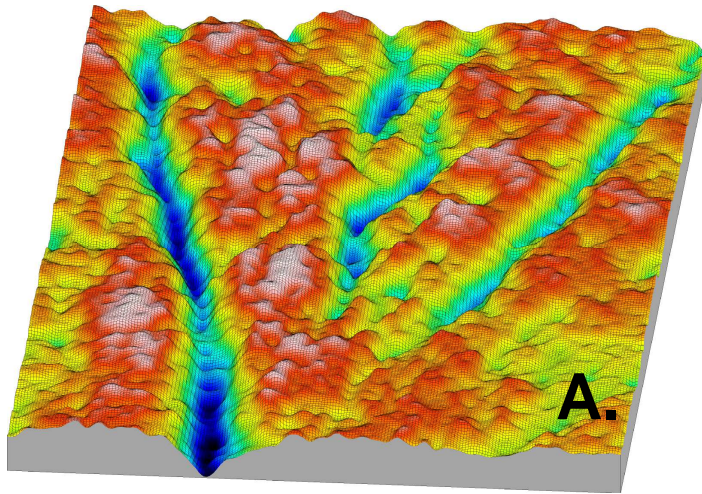
*Phase shifting rapid in vivo measurement of skin (Primos)*



- 1 Projection Unit (Micromirror Array)
- 2 CCD Camera

Principle of Triangulation Combined with Structured Illumination Determines 3D-Profile of Samples (Human Skin)

# Anti-aging efficacy *in vivo*: Crows' feet reduction



- A. Surface at crows' feet area before treatment (t = 0)
- B. Surface after treatment with verum after 28 days

Verum = Formulation containing 0,2% Ectoin and 40 ppm Cyclopeptide-5 encapsulated in a liposome

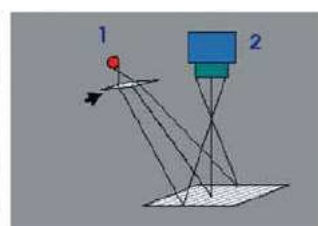
# Anti-Aging efficacy *in vivo*



## Design of clinical study II (Skin Smoothness)

- Surface evaluation by means of Primos at day 0, 14 and 28
- 20 volunteers (n = 20, f) with normal skin
- Female, Age range: 37-63 years (average: 44,8)
- Test area: inner forearm
- application of test products twice daily for 28 days
- Testproducts (verum vs. placebo, 2 mg/cm<sup>2</sup>)

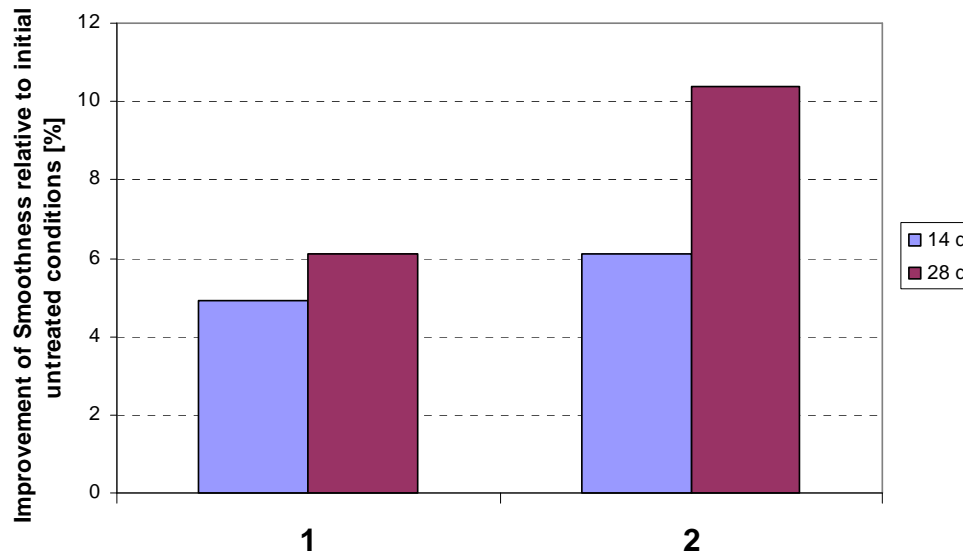
*Phase shifting rapid in vivo measurement of skin (Primos)*



- 1 Projection Unit (Micromirror Array)
- 2 CCD Camera

Principle of Triangulation Combined with Structured Illumination Determines 3D-Profile of Samples (Human Skin)

# Anti-aging efficacy *in vivo*: skin smoothness



1 = Placebo: Formulation without RonaCare® Cyclopeptide-5  
2 = Verum: Formulation with 4% RonaCare® Cyclopeptide-5



**RonaCare® Cyclopeptide-5 improves skin smoothness and leads to wrinkle reduction**

# RonaCare® Cyclopeptide-5



## From a formulators point of view

... due to the flexible cyclohexyl-side-chain of Aminocyclohexane carboxylic acid no crystallization of Cyclopeptide-5 was detected

....Cyclopeptide-5 is an inner salt of high stability. Meaning that this kind of peptide has no need for a counter-ion.

# RonaCare® Cyclopeptide-5



INCI:	Water (for EU: Aqua), Ethanol, Lecithin, (5 %) Ectoin, (100 ppm) Cyclopeptide-5 (INCI applied for)
Appearance:	Opaque, slightly yellowish
Use-level:	2-5%
Formulation hints:	No technical limitations
Applications:	Anti-aging in daily skin care
Article-No:	130198
Pack Sizes:	50 g sample (brown glass bottle) 1 kg (brown glass bottle)
Patent(application)s:	WO 2009/124754, US 6,127,335, EP 904285 B1

Improved availability via liposomal technology