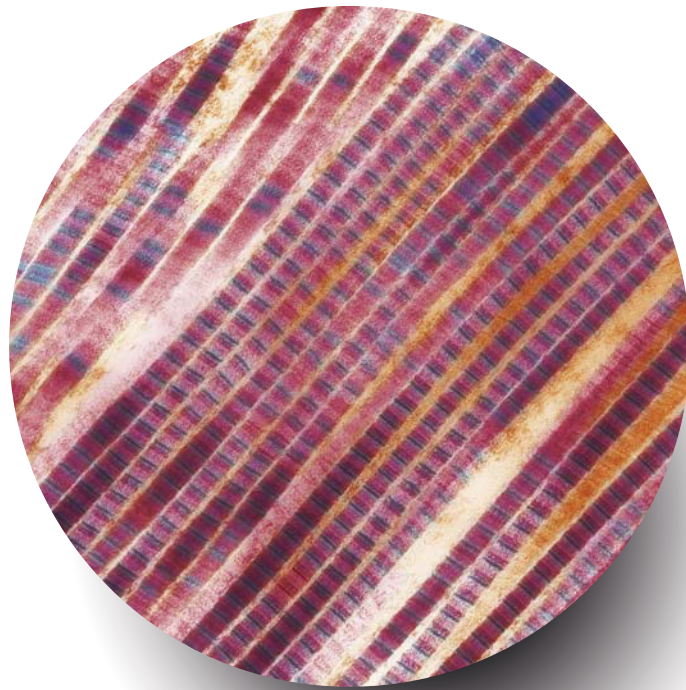


# PEPTIDES IN COSMETICS BACHEM

PIONEERING PARTNER FOR PEPTIDES



# COSMETIC PEPTIDES OFFERED BY BACHEM

Peptides are involved in many physiological processes. Their broad acceptance as natural molecules, relatively high stability and well-defined actions have made them attractive for many skin-related indications, most notably in anti-aging therapy. The aging population and their prevalent wish to keep a young and healthy appearance have substantially driven the development of anti-aging products. Many peptides marketed in cosmetic products are advertised to slow down or even reverse the aging process of the skin. Common modes of action include stimulation of collagen synthesis and inhibition of neurotransmitter release in order to reduce fine lines and wrinkles caused by the repetitive muscular activities of facial expression. We have considerable expertise and long-standing experience in peptide synthesis. With our capacity to upscale the production of simple and modified peptides, we are the partner of choice for the pharmaceutical and cosmetic industries.

## **Introduction**

The cosmetic industry requires a variety of different ingredients for skin care products. These include peptides, growth factors, antioxidants, anti-inflammatory botanicals, and polysaccharides. As these products show drug-like effects they are commonly referred to as cosmeceuticals. In contrast to drugs these ingredients are not regulated by the U.S. Food and Drug Administration (FDA).

Originally, peptides became of interest in cosmetics as a result of the discovery of their beneficial effects in wound healing. As peptides are involved in an immense number of physiological processes, it was logical to further exploit them for cosmetic applications. Most of the peptides used in cosmetics are designed to counteract the

aging process of the skin. The need for such products is driven by the increasing desire in modern society to maintain a young appearance even at an older age, and the breadth of possible treatments to achieve this goal. The increasing knowledge about the molecular details of the skin-related aging processes has significantly contributed to the exploration of novel anti-aging agents.

## **Skin Aging**

Skin aging is a process influenced by extrinsic and intrinsic factors and is manifested by a progressive loss of skin tissue, the gradual loss of skin elasticity, and the appearance of fine lines and wrinkles. Extrinsic factors include exposure to UV-light, environmental pollution, cigarette smoke,

and extreme weather conditions.

The continuous generation of reactive oxygen species (ROS) during cellular metabolism and genetic predispositions are considered as intrinsic factors.

Extrinsic factors, most notably UV irradiation and smoking, seem to be the major contributors to premature skin aging.

Exposure of skin to UV-light increases ROS levels leading to changes in gene and protein structure and function and subsequent skin damage. Obvious effects of UV exposure are the disruption of ongoing collagen synthesis, induction of collagenase and other extracellular matrix protein-degrading enzymes, and the damage of cellular

DNA. The premature skin-aging effects of tobacco smoking are caused by induction of collagenase activity and the reduced blood flow to the skin due to nicotine-induced vasoconstriction. Intrinsic factors such as the generation of ROS contribute to the loss of extracellular matrix proteins, a decrease in cutaneous blood flow, and a loss of cells and cell function.

### Peptides in Cosmetics

Peptides have become very important ingredients in cosmetic products, especially in anti-aging preparations. According to their mode of action, they have been divided into three main groups: signal peptides,

## Custom Synthesis at Bachem

### ✓ Quality

- GMP and non-GMP quality
- Broad range of purities
- State of the art analytical capabilities
- ISO 13485 certified manufacturing site in Vista, USA

### ✓ Chemistry

- Fmoc-, Boc-, Z- and other synthetic strategies
- Native chemical ligation
- Synthesis of complex peptides

### ✓ Capacity

- Production sites in the USA and Europe
- Largest production facilities in the market
- Up-to-date technology

### ✓ Modifications

- Acylation, acetylation, amidation, etc.
- Cyclizations
- Stabilizing modifications

### ✓ Support

- Highly qualified technical support team
- Documentation
- Confidentiality

**Cosmeceutical-related products offered by Bachem**

Prod. No.	Product	Activity
F-1040	Ac-Hyp-OH	Anti-inflammatory, wound healing
E-1135	Ac-Met-OH	Anti-wrinkle
H-1156	Ac-Ser-Asp-Lys-Pro-OH	Stimulation of keratinocyte, fibroblast and follicle dermal papilla cell growth
E-1245	Ac-Tyr-NH <sub>2</sub>	Anti-wrinkle
H-1555	H-Ala-His-Lys-OH	Enhancement of collagen synthesis
G-4555	L-Anserine · nitrate	Antioxidant
G-4425	Carcinine	Antioxidant
G-1250	L-Carnosine	Antioxidant
H-2390	Chemotactic Domain of Elastin (VGVAPG)	Stimulation of skin fibroblast proliferation
H-7316	Dermcidin-1L (human)	Activation of keratinocytes
F-2395	H- $\alpha$ -Difluoro-Me-DL-Orn-OH · HCl · H <sub>2</sub> O	Reduction of excessive hair growth (hirsutism)
H-2750	(D-Ala <sup>2</sup> )-Leu-Enkephalin	Anti-wrinkle peptide
H-3630	H-Gly-Pro-Hyp-OH	Anti-wrinkle and anti-aging
C-1910	Z-Gly-Pro-Phe-Pro-Leu-OH	Inhibitor of desquamation of human skin
H-9795	rec IL-1 $\alpha$ (human)	Role in skin renewal
C-3900	Z-Ile-Glu(OtBu)-Ala-Leu-aldehyde	Inhibitor of accumulation of ubiquitinated proteins in neuronal cells
G-2450	Kyotorphin	Reducing the sensitivity of the skin
H-3510	Liver Cell Growth Factor (GHK)	Enhancement of collagen synthesis
H-4210	H-Lys-Phe-Lys-OH (KFK)	Activation of LAP-TGF- $\beta$
H-1100	(Nle <sup>4</sup> ,D-Phe <sup>7</sup> )- $\alpha$ -MSH	Anti-inflammatory
H-3902	Acetyl-(Nle <sup>4</sup> ,Asp <sup>5</sup> ,D-Phe <sup>7</sup> ,Lys <sup>10</sup> )-cyclo- $\alpha$ -MSH (4-10) amide	Darkening pigmentation in the skin
H-2716	(Met <sup>5</sup> ,Pro <sup>6</sup> ,D-Phe <sup>7</sup> ,D-Trp <sup>9</sup> ,Phe <sup>10</sup> )- $\alpha$ -MSH (5-13)	Anti-inflammatory
H-4230	$\alpha$ -MSH (11-13) (free acid)	Anti-inflammatory
H-6590	(D-Pro <sup>12</sup> )- $\alpha$ -MSH (11-13) (free acid)	Anti-inflammatory
G-2855	H-Phe- $\beta$ -Ala-OH	Inhibitor of hair growth. Eventually promoting effect on wound healing.
H-1592	Procollagen Type I (212-216) (KTTKS)	Enhancement of collagen synthesis
G-3025	H-Pro-Hyp-OH	Stimulation of fibroblast proliferation, chondroprotective
H-7490	rec EGF (human)	Aiding wound healing
H-6920	Rigin (GQPR)	Immunomodulation
H-2472	SPARC (119-122) (mouse)	Stimulation of endothelial cell proliferation and angiogenesis
N-1145	Tfa-Val-Tyr-Val-OH	Inhibitor of elastase
H-2608	Thymosin $\beta_4$ (human, bovine, horse, rat)	Potentially contributing to angiogenesis, wound healing, regulation of inflammation and other processes
N-1170	H-Val-Trp-OH	Peptidic compound in various formulations, e.g. for anti-wrinkle and anti-aging cosmetics
H-5300	H-Val-Tyr-Val-OH	Peptidic compound in various cosmetic formulations

neurotransmitter-affecting peptides, and carrier peptides. The first group mainly consists of peptides which are able to increase collagen synthesis, or alternatively, inhibit the breakdown of collagen by collagenase. The second group includes peptides mimicking the effects of botulinum neurotoxins whereas the third group, the carrier peptides, acts by delivering trace elements required for enzymatic processes.

### Signal peptides

Aged skin is, amongst others, characterized by reduced levels of collagen and elastin. Increasing the number of fibroblasts or their collagen production and/or inhibiting further collagen hydrolysis are therefore considered effective means to halt or slow the aging process of the skin. Many of the peptides used in cosmetic preparations are compounds which act on fibroblasts. One of the peptides described to act in this way is H-Val- Gly-Val-Ala-Pro-Gly-OH (GVGAPG) (Product: H-2390 Chemotactic Domain of Elastin). H-2390 is an elastin-derived peptide sequence repeated several times in tropoelastin. It was found to stimulate the proliferation of human skin fibroblasts presumably via the elastin receptor. The N-terminally palmitoylated peptide is marketed under the name of palmitoyl-oligopeptide and is supposed to penetrate more efficiently through the epidermis than the parent compound.

H-Lys-Thr-Thr-Lys-Ser-OH (KTTKS) (Product: H-1592 Procollagen Type I (212-216)) is a subfragment of the carboxy-terminal propeptide of type I collagen (residues 197-241) and represents the minimal sequence shown to stimulate extracellular matrix biosynthesis in fibroblasts. It augments type I and II collagen and fibronectin production in a dose- and time-dependent manner with no effect on total protein synthesis or on the ratio of secreted proteins to cell-associated proteins. The N-terminally palmitoylated peptide is marketed as a cosmetic ingredient under the designation palmitoyl pentapeptide-3.

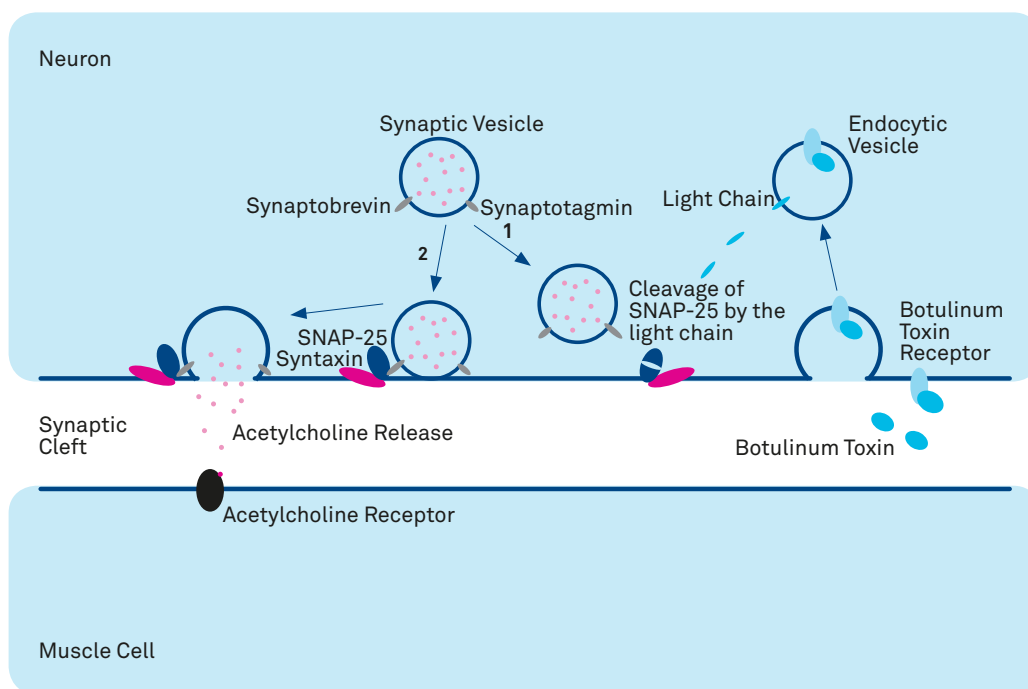
Another peptide shown to enhance collagen production when added to cultured fibroblasts is H-Gly-His- Lys-OH, also known as GHK (Product: H-3510 Liver Cell Growth Factor). Since similar enhance-

ment of collagen synthesis was shown for the tripeptide in complex with copper (see below) it was not clear whether the activity was intrinsic to GHK or whether it was resulting from the formation of active copper complexes due to the presence of copper ions in the tissue culture medium.

As various matrix metalloproteinases are involved in the degradation of collagen and elastin, inhibition of these enzymes represents another strategy to prevent extracellular matrix breakdown and its influence on skin aging. Several peptides have been shown to act in this way: H-Tyr-Tyr-Arg-Ala-Asp-Asp-Ala-OH (YYRADDAA) corresponds to a sequence in  $\alpha$ -1 type I procollagen which is cleaved during the conversion of procollagen to collagen. The peptide was shown to attenuate collagen breakdown by inhibiting procollagen-C proteinase, which cleaves the C-propeptide from type I procollagen. Another compound, the tripeptide H-Lys-Phe-Lys-OH (Product: H-4210) linked to elaidic acid, was demonstrated to activate TGF- $\beta$ , a factor showing the capacity to increase collagen, elastin and TIMP-1 (tissue inhibitor of metalloproteinase 1) expression, and to inhibit MMPs via its lipophilic moiety, elaidic acid. The sequence of H-Lys-Phe-Lys-OH corresponds to the consensus BFB (B: basic amino acid, F: phenylalanine) shown to activate LAP-TGF- $\beta$  in several *in vitro* and *ex vivo* studies. Elaidic acid was chosen due to its inhibitory effect on gelatinase A (MMP-2) and B (MMP-9). Similar effects on TGF- $\beta$  were shown for the palmitoylated peptide Palmitoyl-Lys- Val-Lys-OH and Palmitoyl-Val-Gly-Val-Val-Ala-Pro-Gly-OH, an elastin-derived peptide.

### Neurotransmitter-affecting peptides

Many of the peptides used in cosmetic preparations belong to the group of neurotransmitter-affecting peptides. These peptides act in a similar way as botulinum toxin (Botox). By inhibiting signal transduction pathways at neuromuscular junctions they attenuate the formation of wrinkles and fine lines which appear over time due to the repetitive contraction of the intrinsic muscles of facial expression. Botulinum toxin, synthesized by the bacterium *Clostridium botulinum*, is the most potent toxin known. It is a disulfide-linked heterodimer consisting of a heavy and



### Mode of Action of Botulinum Neurotoxin Type A

Botulinum Neurotoxin Type A consists of a heavy and a light chain. Upon binding to the botulinum toxin receptor the toxin is internalized. Acidification of the vesicles leads to the translocation of the light chain to the cytosol. There it acts as a protease and cleaves the SNARE protein SNAP-25. Cleavage results in inhibition of exocytosis and acetylcholine neurotransmitter release (1) as intact SNAP-25 together with several other proteins is required for synaptic vesicle fusion with the presynaptic membrane (2).

a light chain. Upon binding to the peripheral neuronal presynaptic membrane mediated by the heavy chain the toxin is internalized by receptor-mediated endocytosis. After translocation from the endocytotic vesicle into the cytoplasm, the light chain proteolytically cleaves either SNAP-25 or synaptobrevin depending on the serological subtype of the neurotoxin. Cleavage of these proteins which are essential for docking to and fusion of acetylcholine vesicles with the inner side of the nerve terminal membrane results in the inhibition of neurotransmitter release at neuromuscular junctions. A well-known mimic of botulinum toxin used in cosmetic preparations is Acetyl-Glu-Glu-Met-Gln-Arg-Arg-NH<sub>2</sub>. It is a synthetic peptide corresponding to a sequence within the N-terminal region of SNAP-25 (amino acids 12-17) and competes with SNAP-25 for a position in the SNARE

complex, thereby modulating its formation. The resulting destabilization of the SNARE complex leads to an inhibition of neurotransmitter release and a subsequent attenuation of muscle contraction.

Another class of potentially interesting substances in the cosmetic industry includes peptides derived from snake venoms. Waglerin-1 (H-Gly-Gly-Lys-Pro-Asp-Leu-Arg-Pro-Cys-His-Pro-Pro-Cys-His-Tyr-Ile-Pro-Arg-Pro-Lys-Pro-Arg-OH), for example, is a peptide isolated from the venom of the temple viper, *Tropidolaemus wagleri*. It consists of 22 amino acids and selectively blocks the epsilon form of the muscular nicotinic acetylcholine receptor (mnAChR) thereby keeping the affected muscles in a relaxed state. Another compound, the tripeptide H-β-Ala-Pro-Dab-NHBzl mimicking

the activity of waglerin-1 is now marketed in anti-wrinkle creams.

Many other peptides acting similarly by affecting neurotransmitter release are promoted by the cosmetic industry. Compounds include: a) a pentapeptide, which binds to the enkephalin receptors on the outside of neurons and thereby initiates a cascade leading to a decrease in acetylcholine release and subsequent muscle contraction; b) SNAP-8, an octapeptide related to and acting in the same way as Acetyl-Glu-Glu-Met-Gln-Arg-Arg-NH<sub>2</sub>; and c) a hexapeptide that reduces the activation of muscle-specific kinase (MuSK) by blocking the agrin binding site. The peptide was demonstrated to disrupt acetylcholine receptor clustering, a requirement for acetylcholine to trigger the signal for muscle contraction.

#### **Carrier peptides**

The tripeptide H-Gly-His-Lys-OH (GHK) (Product: H-3510 Liver Cell Growth Factor) was originally identified in human plasma and has a high affinity for copper<sup>2+</sup> (Cu<sup>2+</sup>). It acts as a signaling peptide and a carrier molecule for copper which is a co-factor for several enzymes involved in collagen and elastin formation. The copper peptide was shown to stimulate wound healing but also to reduce fine lines and wrinkles and to improve elasticity and firmness of aged skin. A wide variety of effects have been ascribed to GHK-Cu. The peptide exhibits anti-inflammatory actions by suppressing the expression of pro-inflammatory cytokines. It also chemoattracts capillary cells, macrophages and mast cells, increases the synthesis of collagen and elastin, and stimulates the proliferation of fibroblasts and keratinocytes.

#### **Other mechanisms**

Many peptides used in cosmetic preparations act by other mechanisms to improve skin appearance or delay skin aging. These include ROS scavengers, collagen fiber organizing compounds, and anti-inflammatory peptides: Peptides such as carnosine (Product: G-1250), anserine (Product: G-4555 L-Anserine · nitrate) and carbinine (Product: G-4425) are histidine dipeptides with antioxidant activity. Carnosine has been shown to scavenge ROS and che-

late prooxidative metals. It also inactivates reactive mono- and dialdehydes released during the oxidative breakdown of unsaturated lipids thereby protecting hydrophilic and lipophilic biological molecules from oxidative damage. However, in contrast to carbinine it is sensitive to enzymatic hydrolysis by carnosinase. A tetrapeptide of unpublished sequence is a mimic of decorin which interacts with collagen and influences collagen fibrillogenesis. As functional decorin in the skin diminishes with age, the tetrapeptide is proposed to serve as a substitute. By controlling collagen aggregation and homogenization of fibril diameters and dimensions, it increases skin suppleness and thus improving its appearance. Rigin (Product: H-6920 H-Gly-Gln-Pro-Arg-OH (GQPR)) corresponds to amino acid sequence 341- 344 of the human IgG H-chain and has been shown to possess immunomodulatory activity. By suppressing inflammation the palmitoylated form is supposed to accelerate tissue repair thereby leading to increased skin firmness, smoothness, and elasticity.

#### **Conclusions**

Synthetic peptides have become important for the cosmetic industry. Due to demographic changes and the aging population wishing to maintain a young lifestyle and appearance, cosmetic industry research has focused on anti-aging skin therapy. Today, more than 25 peptides are used in anti-aging skin care products and there are many more in development.

Bachem is the market leader in the production of synthetic peptides and has long standing partnerships with major firms in the pharmaceutical and cosmetic industry. We are well-equipped for small to industrial scale synthesis of peptides of any complexity. Bachem is therefore the ideal partner in the development and production of cosmetic peptides.

For further details, please see literature references.





## COLLAGEN FIBRES

Colored transmission electron micrograph (TEM) of a section through healthy collagen fibres from human skin. Collagen is the major structural protein in the body, forming a large part of bones, tendons and tissues. Magnification unknown.

KEYSTONE/SCIENCE PHOTO LIBRARY/  
STEVE GSCHMEISSNER



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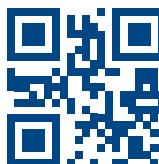
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