Personal Care

Protein technology

Rob Sayer
R&T Manager
Croda

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Innovation you can build on™

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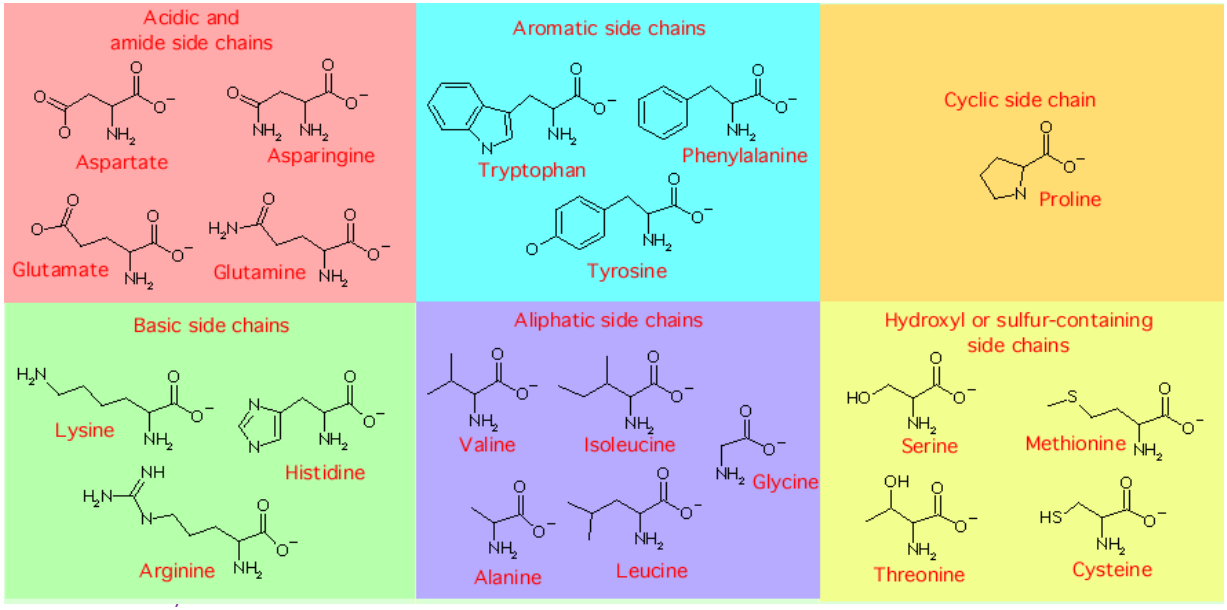
Proteins - What Are They?

- Proteins are complex nitrogen-containing natural products made up of amino acids of varying chemical composition
- Proteins may be processed to create personal care ingredients that provide impressive functional benefits from natural derivation



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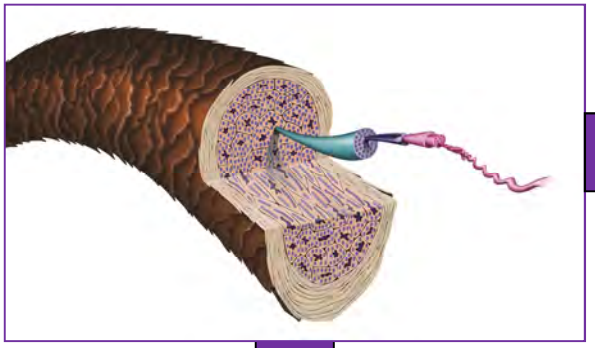
Proteomics: Hair Fibre and Products



- Structure / function correlation
- Natural variance (age, location, demographics)
- Ethnic variance

Damage
Shampoo, Conditioners, Heat, UV, Bleach, Pollution, Relaxing, Colouring… … …

Effects of hair fibre
- Proteins damaged
- Damage sites
- Cortex v's cuticle
- Protein function affected

Proteins leached
- Proteins lost
- Cortex v's cuticle
- Protein function

Raw Material / Cosmetic Product
- Amino acid composition
- Peptide length
- Peptide sequence
- Protein function
- Cross match with hair peptides

Amino Acid Profiles – High Percentage

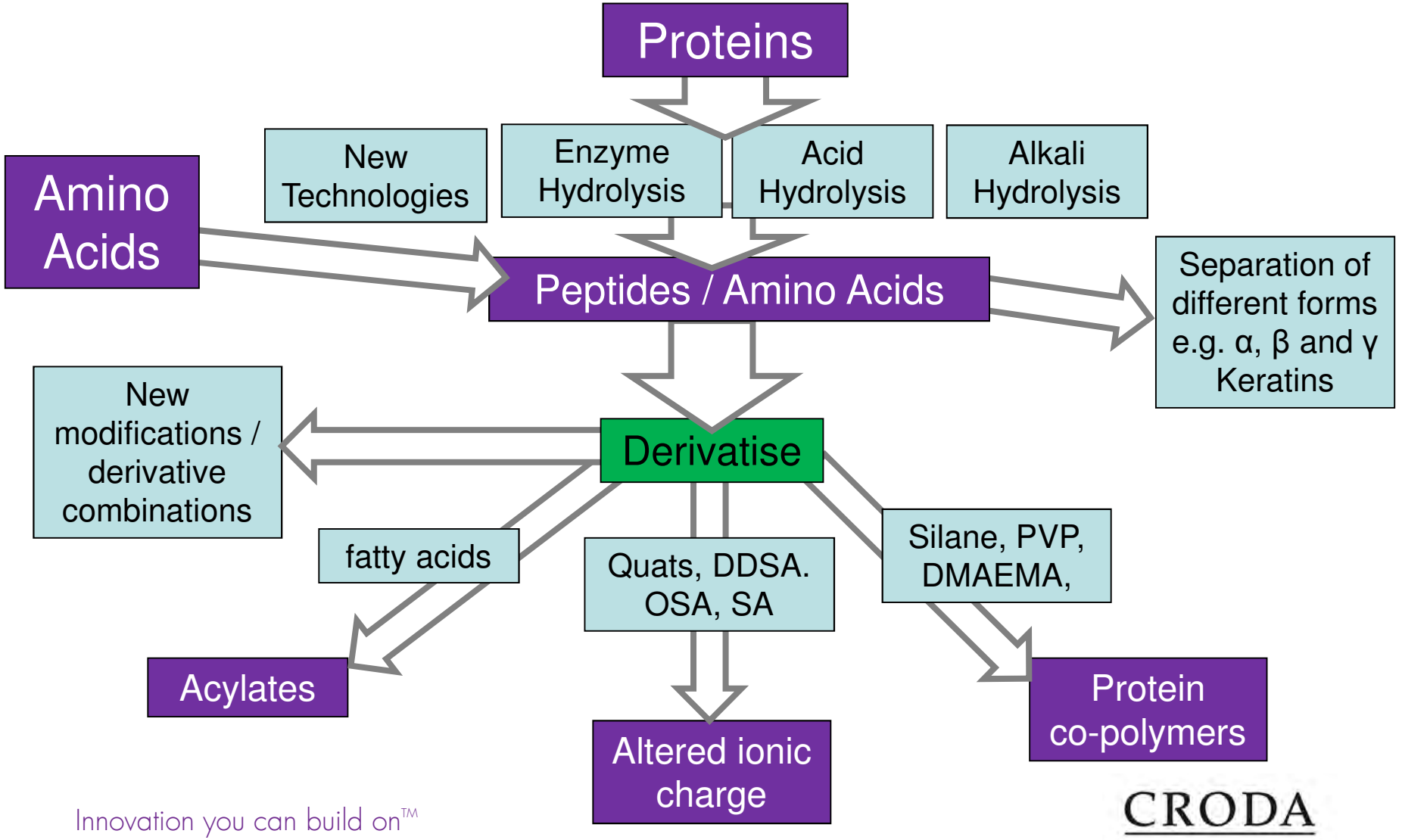
Amino Acid (% w/w)	Wheat	Sesame	Lupin	Apricot	Brazil Nut	Silk	Potato	Collagen	Wool
Alanine	2.7	6.0	4.7	5.1	3.9	**28.5**	5.0	10.2	4.1
Arginine	3.2	**13.9**	9.4	8.4	**14.8**	1.5	5.2	9.2	6.3
Aspartic acid	3.1	**10.0**	**14.4**	**14.8**	8.9	4.7	**12.1**	6.7	5.3
Cystine	**1.8**	0.0	0.3	0.0	**1.8**	0.1	1.2	0.0	**16.7**
Glutamic acid	**36.7**	**24.3**	**28.6**	**30.3**	**17.7**	4.1	**13.6**	11.6	**12.6**
Glycine	3.5	6.9	5.7	5.9	5.0	**34.7**	4.3	**24.3**	5.8
Histidine	2.2	2.8	2.9	3.1	2.7	0.8	2.7	0.8	0.8
Isoleucine	3.4	2.9	2.2	3.1	3.1	0.8	4.3	1.7	2.6
Leucine	7.3	4.8	2.3	5.7	7.5	1.2	9.6	3.4	5.9
Lysine	1.7	3.0	6.6	2.5	3.1	1.4	7.7	4.5	2.4
Methionine	1.5	2.6	0.2	0.6	7.2	0.2	2.2	0.9	0.5
Phenylalanine	5.4	3.5	1.4	3.1	3.7	0.9	4.7	2.3	1.7
Proline	**11.9**	4.4	5.8	5.3	4.7	1.2	5.4	**16.0**	8.2
Serine	5.7	5.3	6.3	4.2	5.1	**15.4**	6.1	4.1	**12.0**
Threonine	2.9	4.3	4.6	2.7	3.1	1.9	6.1	2.2	7.4
Tyrosine	2.9	0.0	0.1	0.1	2.9	0.6	4.9	0.3	2.1
Valine	4.1	5.3	4.5	5.1	4.8	2.0	5.5	2.6	5.4
Hydroxylysine	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0
Hydroxyproline	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**14.2**	0.0

Amino Acid Profiles – Differentiation

Amino Acid (% w/w)	Wheat	Sesame	Lupin	Apricot	Brazil Nut	Silk	Potato	Collagen	Wool
Alanine	2.7	6.0	4.7	5.1	3.9	**28.5**	5.0	10.2	4.1
Arginine	3.2	**13.9**	9.4	8.4	**14.8**	1.5	5.2	9.2	6.3
Aspartic acid	3.1	10.0	**14.4**	**14.8**	8.9	4.7	12.1	6.7	5.3
Cystine	**1.8**	0.0	0.3	0.0	**1.8**	0.1	**1.2**	0.0	**16.7**
Glutamic acid	**36.7**	24.3	28.6	30.3	17.7	4.1	13.6	11.6	12.6
Glycine	3.5	6.9	5.7	5.9	5.0	34.7	4.3	**24.3**	5.8
Histidine	2.2	2.8	2.9	3.1	2.7	0.8	2.7	0.8	0.8
Isoleucine	3.4	2.9	2.2	3.1	3.1	0.8	4.3	1.7	2.6
Leucine	7.3	4.8	2.3	5.7	7.5	1.2	9.6	3.4	5.9
Lysine	1.7	**3.0**	6.6	2.5	**3.1**	**1.4**	7.7	4.5	2.4
Methionine	1.5	2.6	0.2	0.6	7.2	0.2	2.2	0.9	0.5
Phenylalanine	5.4	3.5	1.4	3.1	3.7	0.9	4.7	2.3	1.7
Proline	11.9	4.4	5.8	5.3	4.7	1.2	5.4	16.0	8.2
Serine	5.7	5.3	6.3	4.2	5.1	**15.4**	6.1	4.1	**12.0**
Threonine	2.9	4.3	4.6	2.7	3.1	1.9	6.1	2.2	7.4
Tyrosine	2.9	0.0	0.1	0.1	2.9	0.6	4.9	0.3	2.1
Valine	4.1	5.3	4.5	5.1	4.8	2.0	5.5	2.6	5.4
Hydroxylysine	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**1.0**	0.0
Hydroxyproline	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**14.2**	0.0

Brazil Nut / Silk – high cationic content, Wool – high cystine content,
Collagen – high hydroxyproline content

Proteins


Proteins
Amino Acids
New Technologies
Enzyme Hydrolysis
Acid Hydrolysis
Alkali Hydrolysis
Peptides / Amino Acids
Separation of different forms e.g. α, β and γ Keratins
New modifications / derivative combinations
Derivatise
fatty acids
Quats, DDSA. OSA, SA
Silane, PVP, DMAEMA,
Acylates
Altered ionic charge
Protein co-polymers

Effect of Hydrolysis

- The hydrolysis process can alter the amino acid composition.
- The acid treatment in this case is the most harsh and degrades the cystine and tyrosine
- This alteration could alter the claims that can be made for the resultant cosmetic product

Amino Acid (% w/w)	WHEAT		
	Alkali	Acid	Enzyme
Alanine	2.7	2.8	2.1
Arginine	3.2	3.3	3.2
Aspartic Acid	3.1	3.1	2.8
Cystine	1.8	**0.2**	1.2
Glutamic Acid	36.8	38.3	34.0
Glycine	3.5	3.6	3.4
Histidine	2.2	2.3	2.2
Isoleucine	3.4	3.5	3.4
Leucine	7.3	7.6	6.9
Lysine	1.7	1.8	1.2
Methionine	1.5	1.6	1.5
Phenylalanine	5.4	5.6	5.7
Proline	12.0	12.5	12.9
Serine	5.7	5.9	5.7
Threonine	2.9	3.0	1.5
Tyrosine	2.9	**0.3**	3.1
Valine	4.1	4.3	3.9

‘Brief’ Case Studies

- Underived Peptides - Hydrolysates
  - Peptides and amino acids - Cortex
- Altering the Ionic Character – Quaternary Derivatives
  - Cuticle
- Altering the Mode of Action – Tuneable Silane Derivatives
  - Cortex and Cuticle
- Combining Derivatives: Quaternisation plus Silanation
  - An example from a home care application

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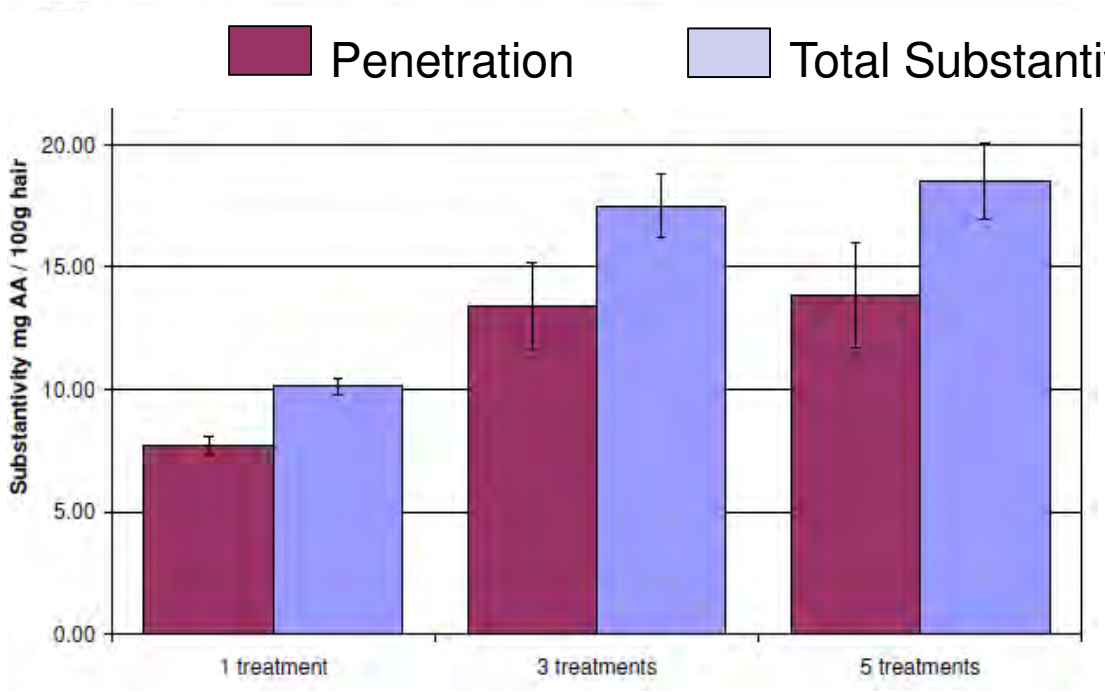
Underived Hydrolysates

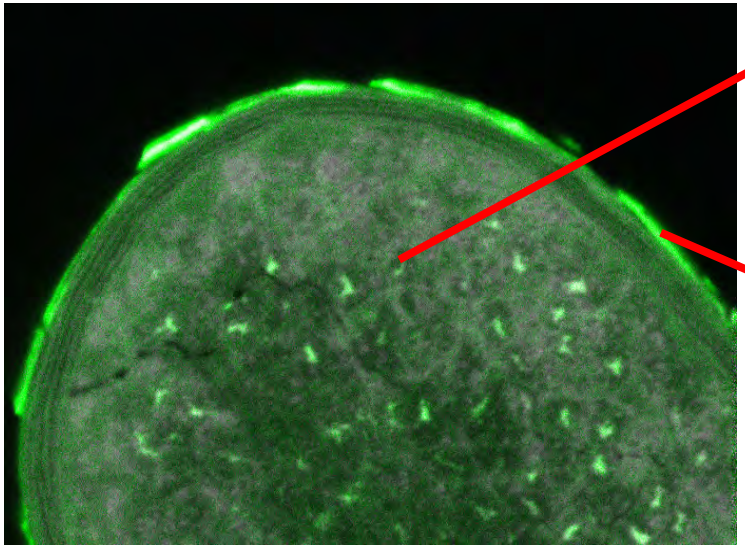
- It has been reported by Kamath, and Robbins that molecular weights of polypetpdies (100 Daltons) and larger species (e.g. molecular weights of up to 10,000) can penetrate into the cortex.
- Radiolabelling studies (carbon and Iodine isotopes) can be used to differentiate between substantivity and penetration



Silk Amino Acids
(1 % active)
Show internal and
surface components

Visualising Uptake

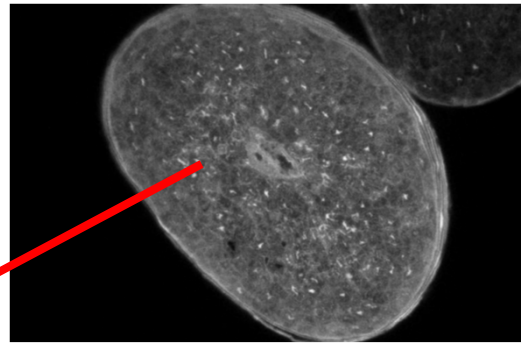
- The protein distribution within the hair can be imaged using confocal fluorescence Spectroscopy.
- This highlights the penetration of the peptides throughout the cortex and any surface components



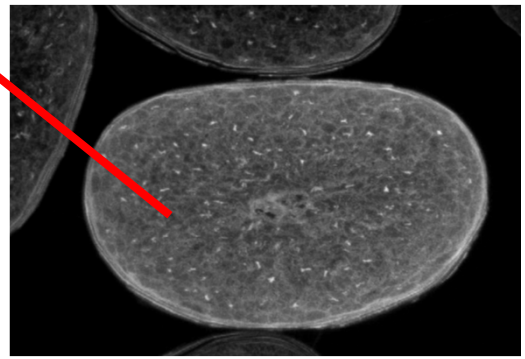
Aqueous treatment of a multi functional protein product

Penetrating protein

Surface active protein



*1 * 1 minute Conditioner Wash* ***Potato*** *hydrolysate*



*3 * 1 minute Conditioner Washes* ***Potato*** *hydrolysate*

Cortex – Water Binding

- As the high molecular weight parent protein is hydrolysed more of the peptide chains are cleaved resulting in an increase in the N- and C- terminated moieties. This increases the hydrogen bonding capability of the peptides and results in more water binding capacity.

- Kyte and Doolittle created a simple system known as the hydropathy indexfor discussing the hydrophobic and hydrophillic nature of amino acids

Source	% Hydrophobic	% Hydrophilic	% Neutral
Wheat	26.2	58.8	15.0
Potato	31.9	46.7	21.4
Lupin	15.6	67.7	16.7
Apricot	22.7	64.4	12.9
Sesame	25.1	58.4	16.5
Brazil Nut	32	51.9	16.1
		Highest	Usually lowest

Amino Acid	Nature
Ile	most hydrophobic
Val	
Leu	
Phen	
Cys	
Met, Ala	
Gly	Neutral
Thr, Ser	
Trp, Tyr	
Pro	most hydrophillic
His	
Asn, Gln	
Asp, Glu	
Lys	
Arg	

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Uptake of Moisture – The Active

The samples were placed in the humidity cabinet set at 50% relative humidity (RH) and then in a dessicator at a RH of 18.8%.

$$\% \text{ Moisture Uptake} = \frac{W_B - W_A}{W_A} \times 100$$

	Glycerine	Potato Peptide	Wheat peptide
% Moisture Uptake (50 % RH)	41.2	102.5	89.3
% Moisture Uptake (18 % RH)	16.3	91.1	78.8
% Water Loss	24.8	11.4	10.4

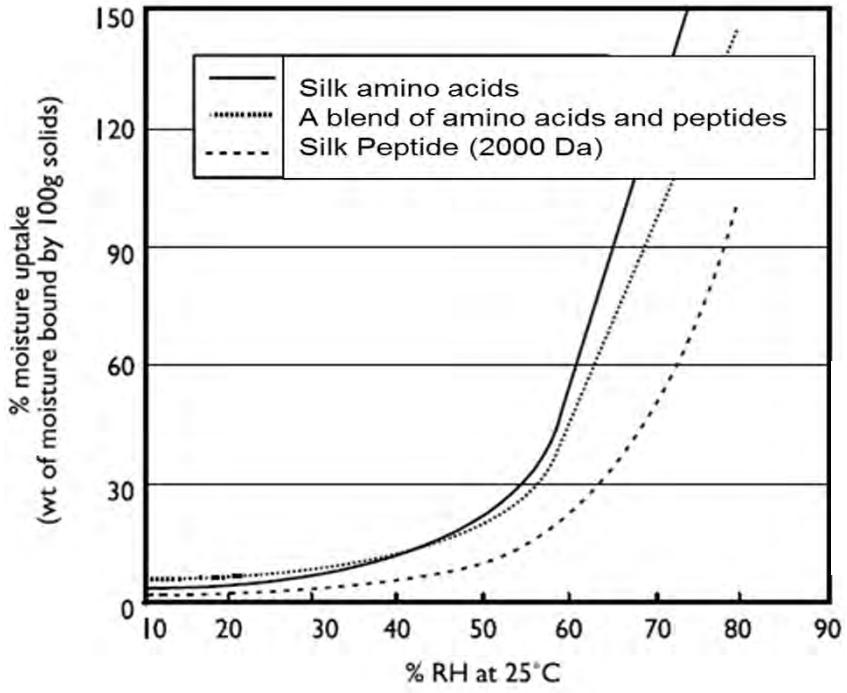
The study shows that the peptides have greater uptake of water and greater water retention than glycerine.

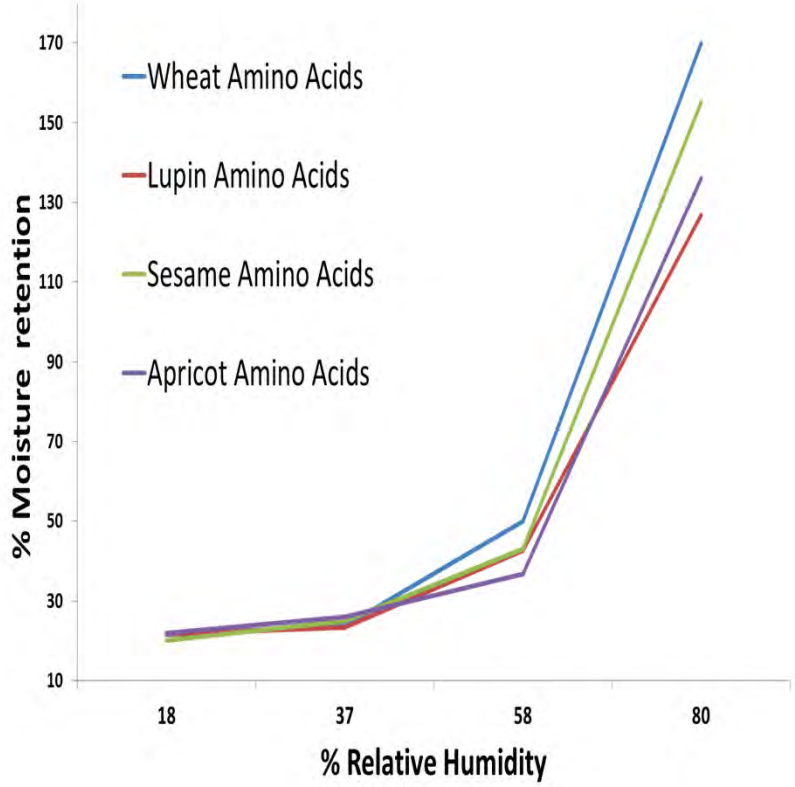
This cannot be attributed to the hydropthy index alone and is determined by: protein source, amino acid profile before and after hydrolysis, sample charge characteristics, molecular weight distribution ... ...

Uptake of Moisture – The Active

Protein sources show varying uptake of moisture

This is even evident when the source is hydrolysed down to amino acids





Varying molecular weights of proteins show a variation in the uptake of moisture.

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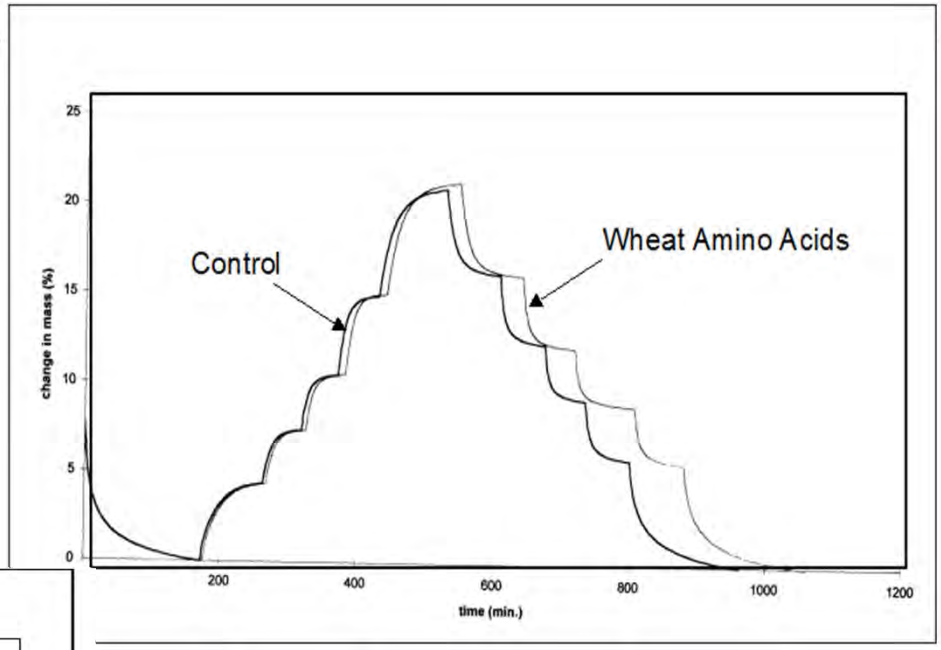
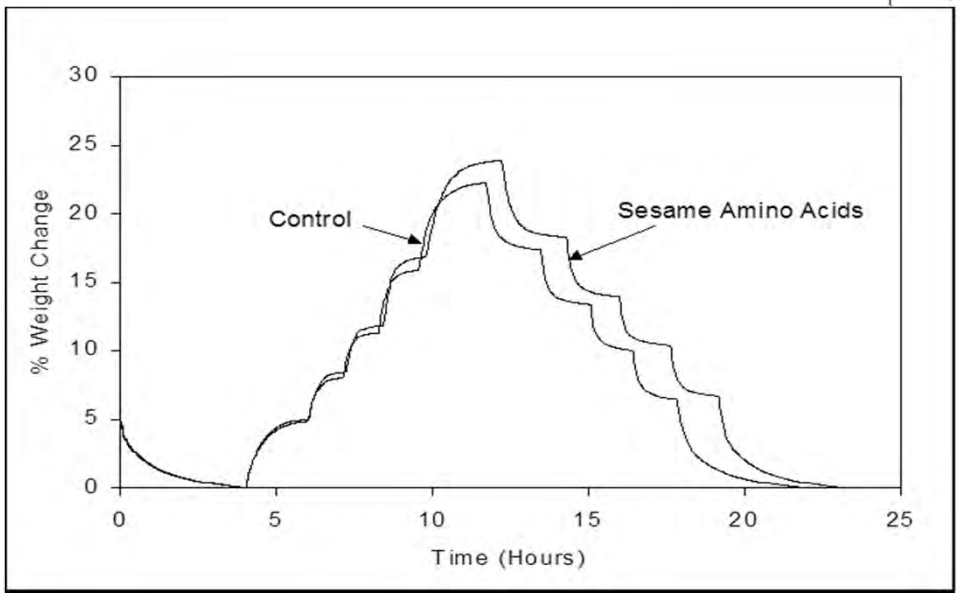
Moisture Kinetics - DVS

- DVS is used to study kinetic moisture properties of protein treatments on hair fibres
- Different sources show differing behaviour for water uptake and retention on hair tresses (1 % active)





Sesame amino acids show greater moisture uptake at equilibrium

wheat amino acids show a greater moisture retention

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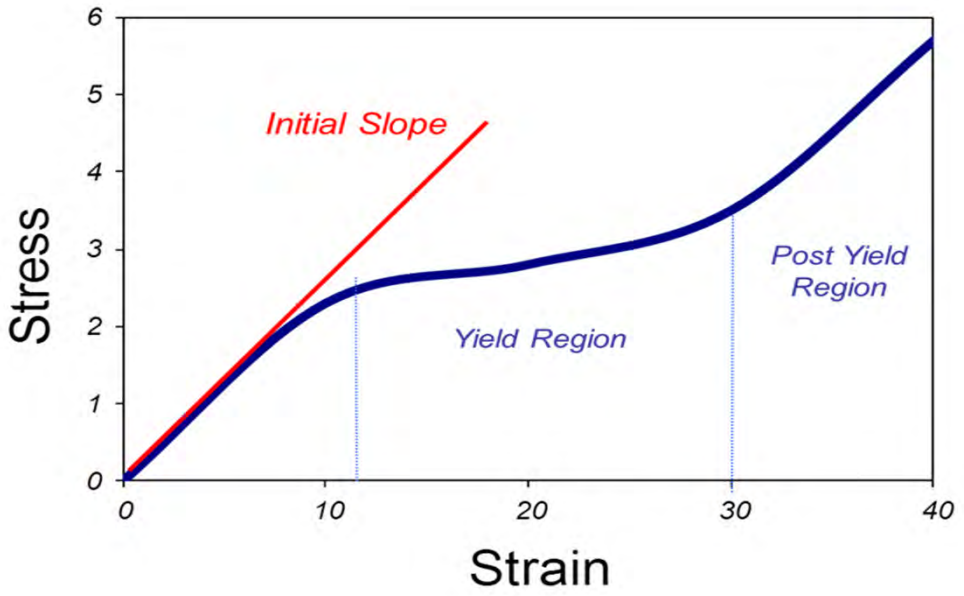
Effects of Protein Penetration

- Proteins penetration into the cortex acts to alter the water binding properties. This can be measured through tensile measurements of hair fibres



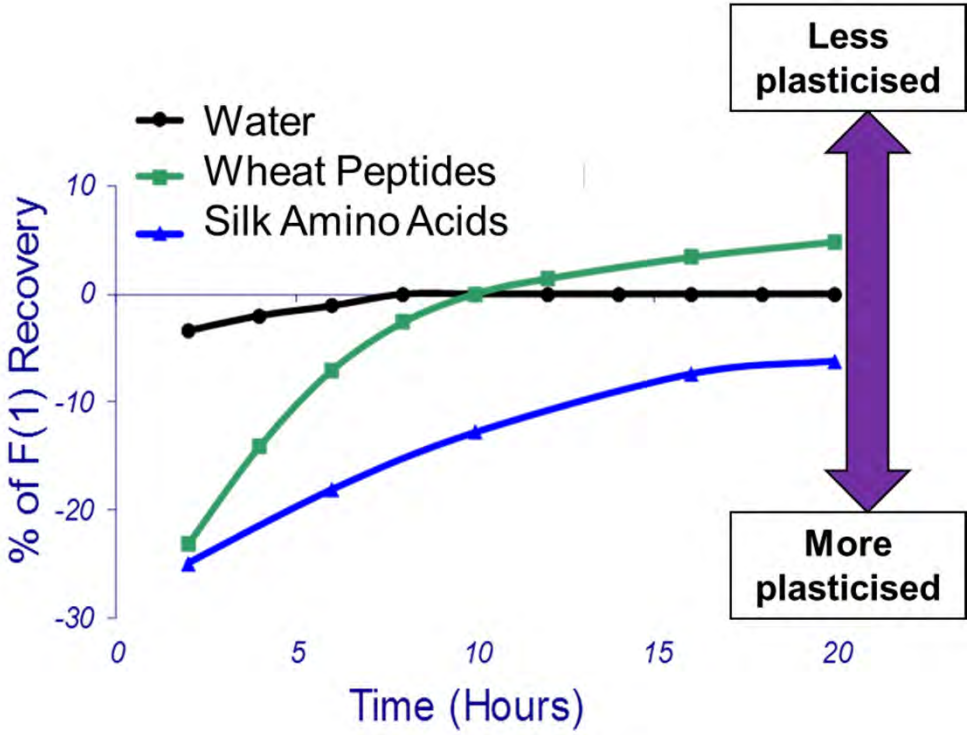
- Proteins can moderate the Young's modulus over a range of relative humidities demonstrating moisture regulating properties. Treatment causes hair to be less prone to the effects of high and low humidity improving the hairs manageability and style maintenance.

Tensile Testing F(1)

F(1) is the force required along the hair fibre to stretch it by 1 %.

The recovery of F(1) over time looks at the plasticity of the hair fibre

Aqueous treatment initially allows the water to plasticise the hair. The force required is reduced then recovers back to the equilibrium value



Wheat peptides show initial plasticisation but then a strengthening effect as the force required becomes greater than the equilibrium value

Silk amino acids retain the moisture and retain the plasticity over time to equilibrium

Hydrolysates - Summary

- Protein sources can be hydrolysed to create short chain peptides or amino acids which can penetrate into the hair fibre altering the moisture properties of the cortex
- The effects are dependent on both the molecular weight of the product and also the amino acid distribution
- Techniques assess the uptake, water binding properties and also the effect the product has on the fibre through tensile measurement.
- These have shown that proteins can mediate the water properties of the hair fibre resulting in more manageable, and plasticised hair

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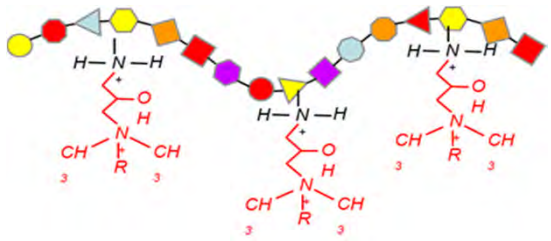
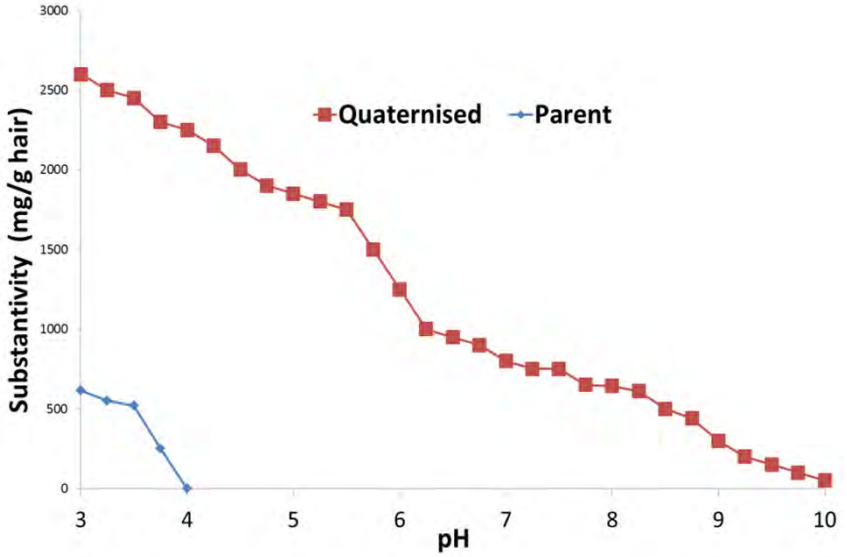
Quaternised Derivatives



- Altering the ionic charge alters the iso-electric point of the peptide.

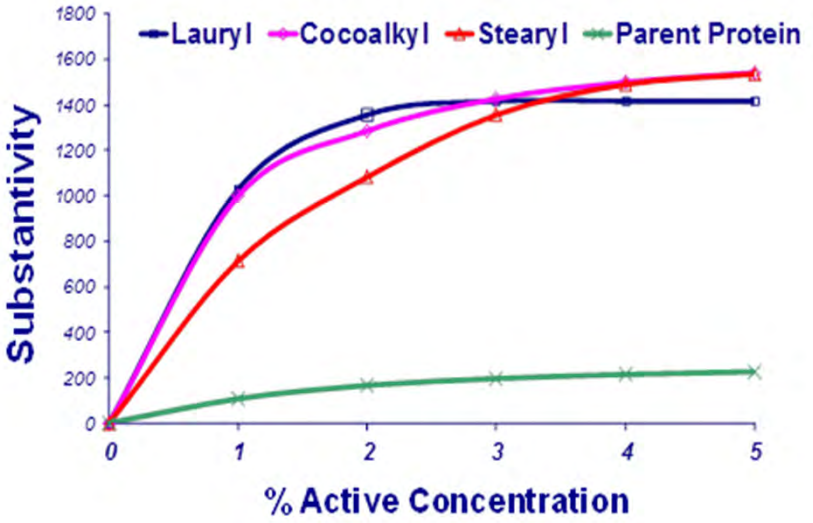
  Wheat peptides have an IEP of 4

  Quaternised wheat peptides have an IEP of 10

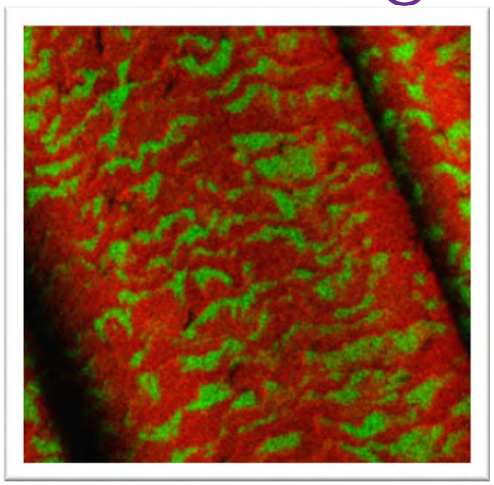


- At any pH below 10 the quaternised wheat peptides will be positively charged and therefore substantive to the hair surface

- The quaternary functionality can be used to deliver different fatty acid chain lengths to the hair fibre. The fatty acid chain length has very little effect on the substantivity but can affect the conditioning performance and sensory properties

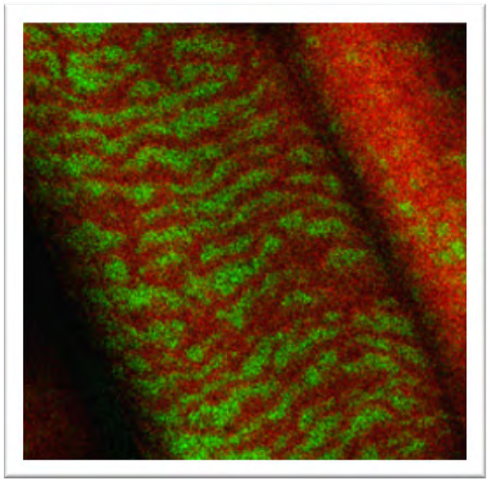


Visualising Protein Quat Deposition on Hair



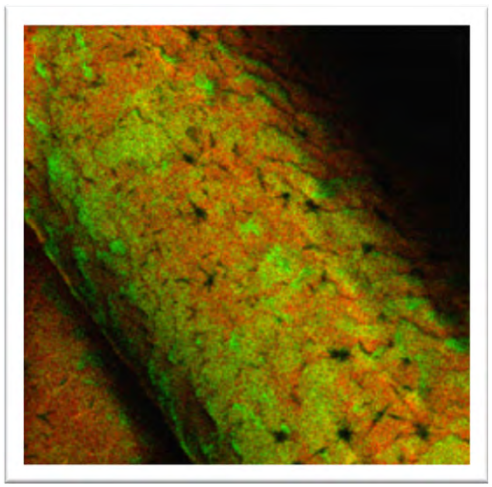
Root

Cuticle damage and cuticle ageing lead the raised edges becoming anionic in nature. The keratin quat deposits most strongly to these areas



Middle

Cuticle edge damage is more apparent and the damage forms lines around the hair fibre. The deposition in green shows the lines of damage

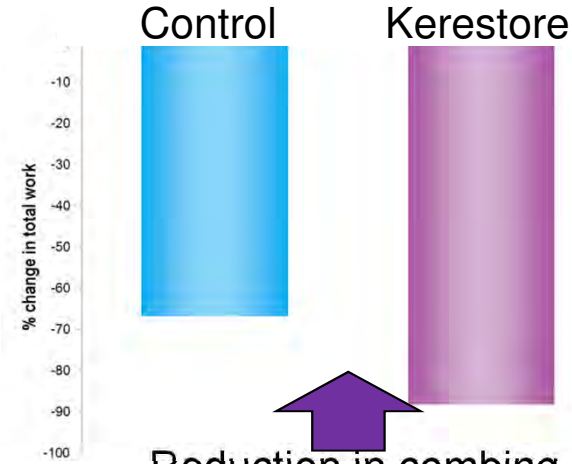


Tip

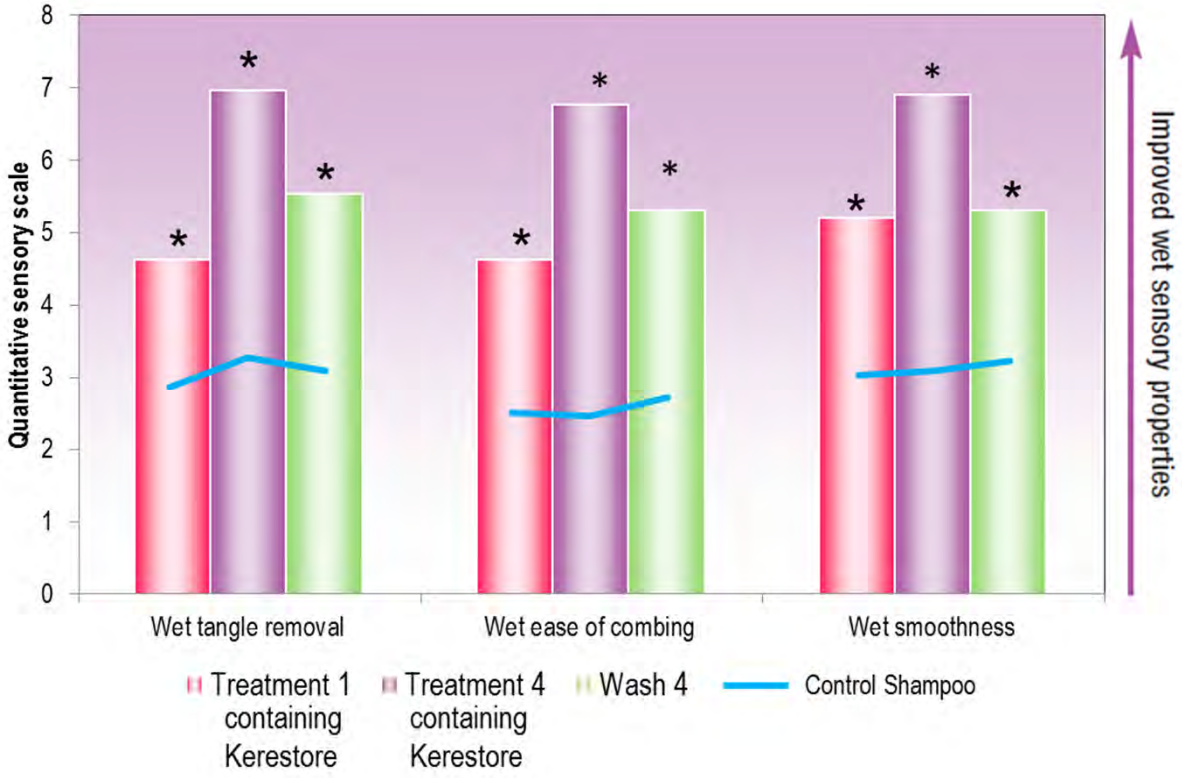
As the cuticles are worn away at the tip of the hair fibre. The keratin quat deposits all over the hair fibre but is more concentrated in certain areas showing the most damage

Benefits of Protein Quats

- Consumer perceivable benefits can be shown through combing studies from a conditioner base and conditioning from a shampoo base through sensory panel studies



Reduction in combing force for bleached European tresses from a conditioner base (**1 % Active**)



Conditioning study by sensory panel from a shampoo base (**0.2% active**)

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Quaternisation Summary

- Adding cationic nature means that they are substantive to the outside of the hair fibre.
- They are substantive from a range of formulation types at low active concentrations and varying pH's when compared to the parent proteins.
- The molecular weight, fatty acid and amount or combination of quats can affect the end properties of the product and the effect on the hair
- By combining knowledge of hydrolysis and quaternisation together a bio-mimic can be created offering targeted repair and conditioning from a shampoo system

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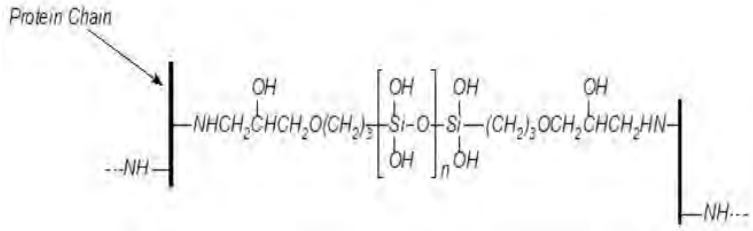
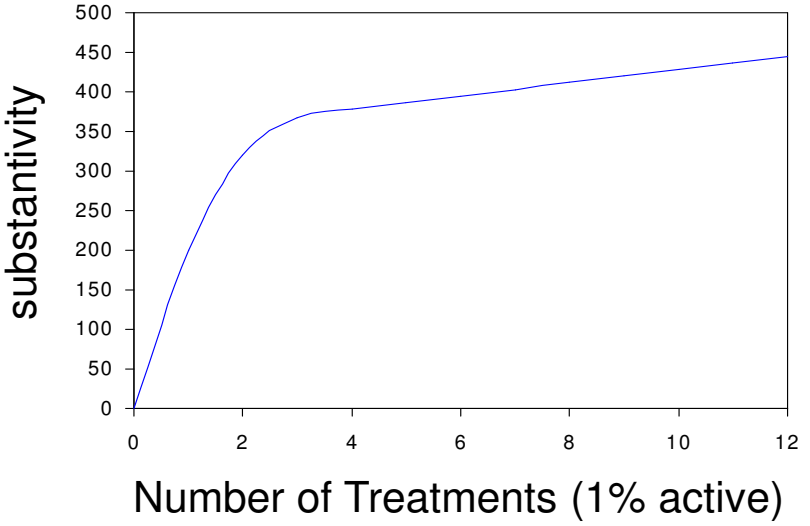
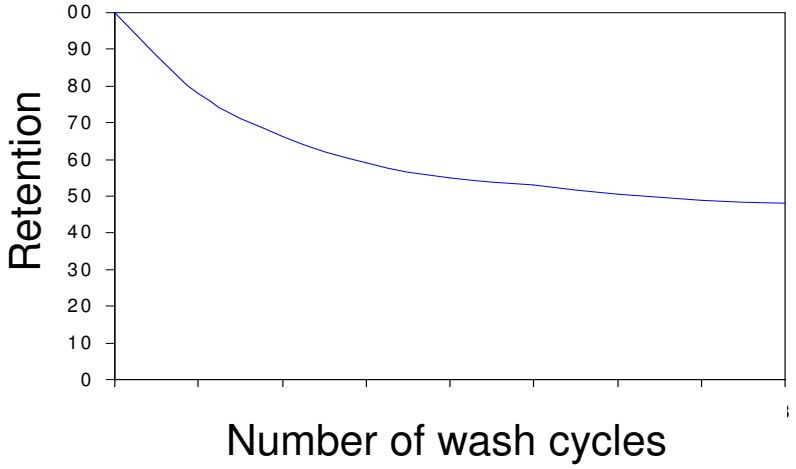
Silane Derivatives



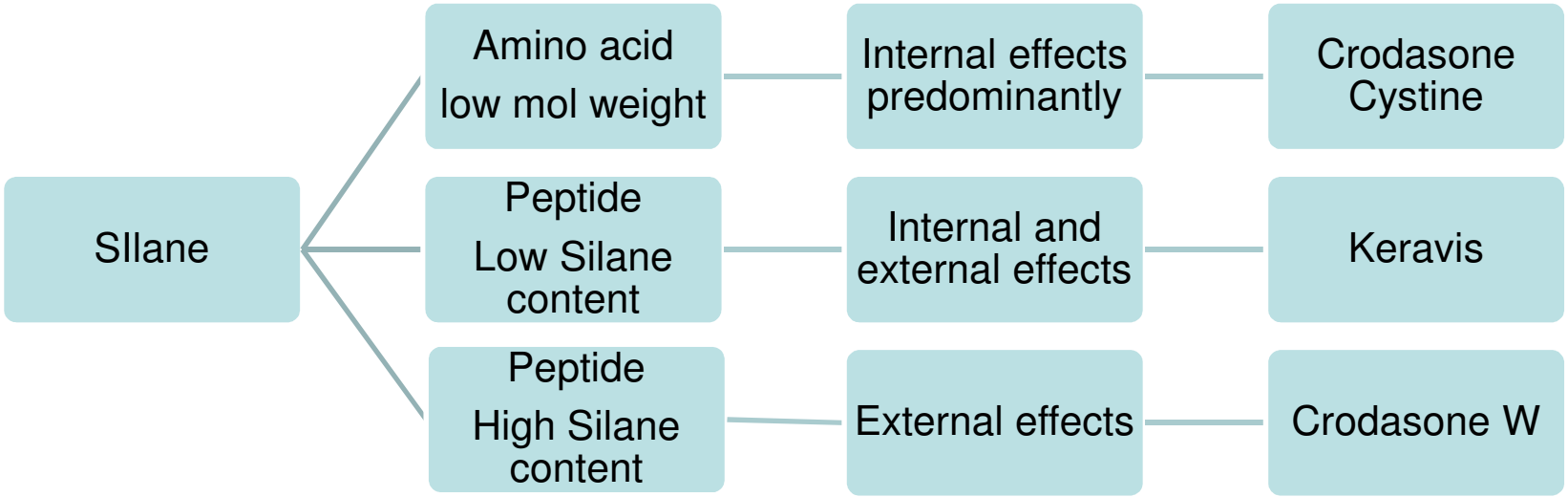
- Peptides and amino acids can be derivatised using silanes
- These chemically bond to the amino groups on the peptide
- There effect is that they can be used to impart heat activated cross-linking functionality
- They shows good resistance to washing cycles





Silane Technologies

- Applying silanes to different base peptides can impart different end functionality. The effects can be further altered by changing the level of substitution applied



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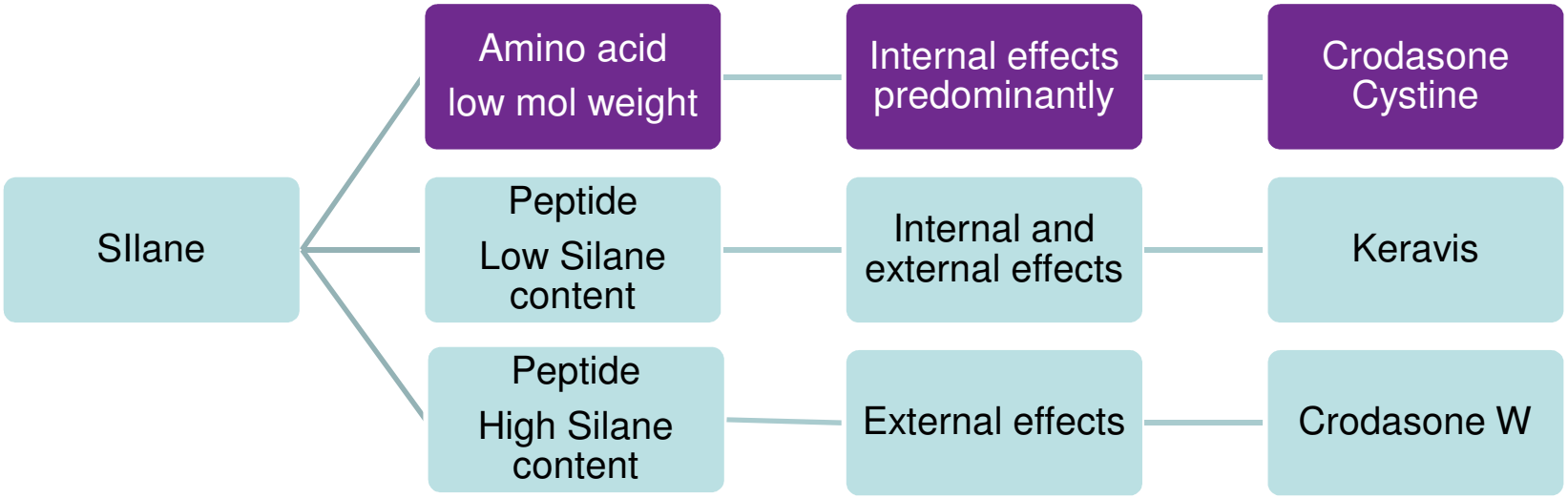
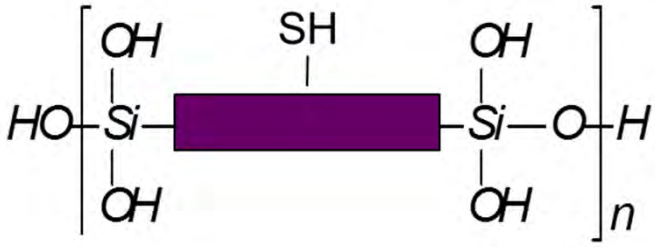
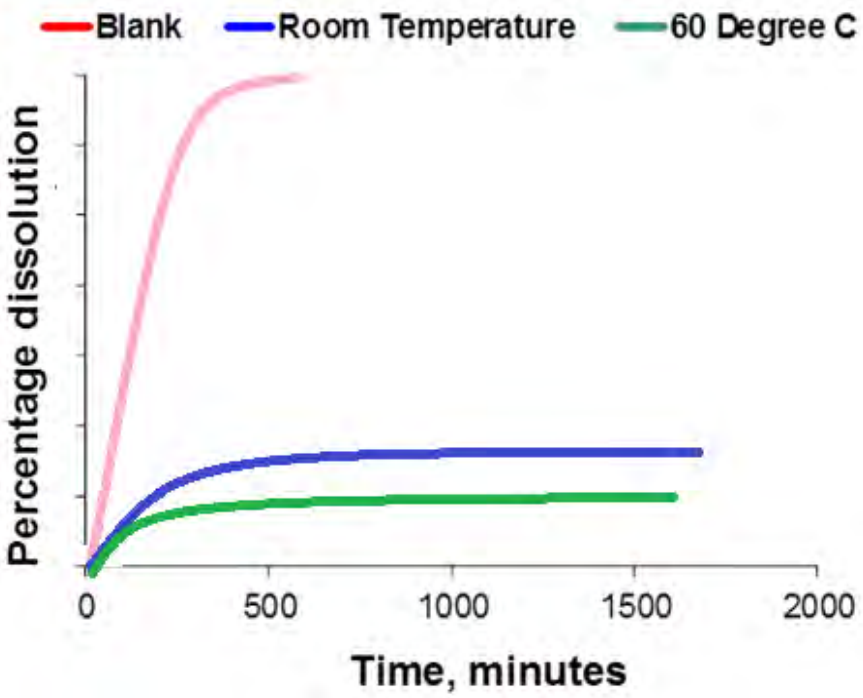


Silanated Cystine

- Cysteine is a single amino acid
- The hair is naturally rich in cysteine
- We can take advantage of the SH group – to form covalent bonds with the cystine in the hair fibres
- We can silanate the amino acid adding the cross-linking functionality
- This adds a heat activation claim

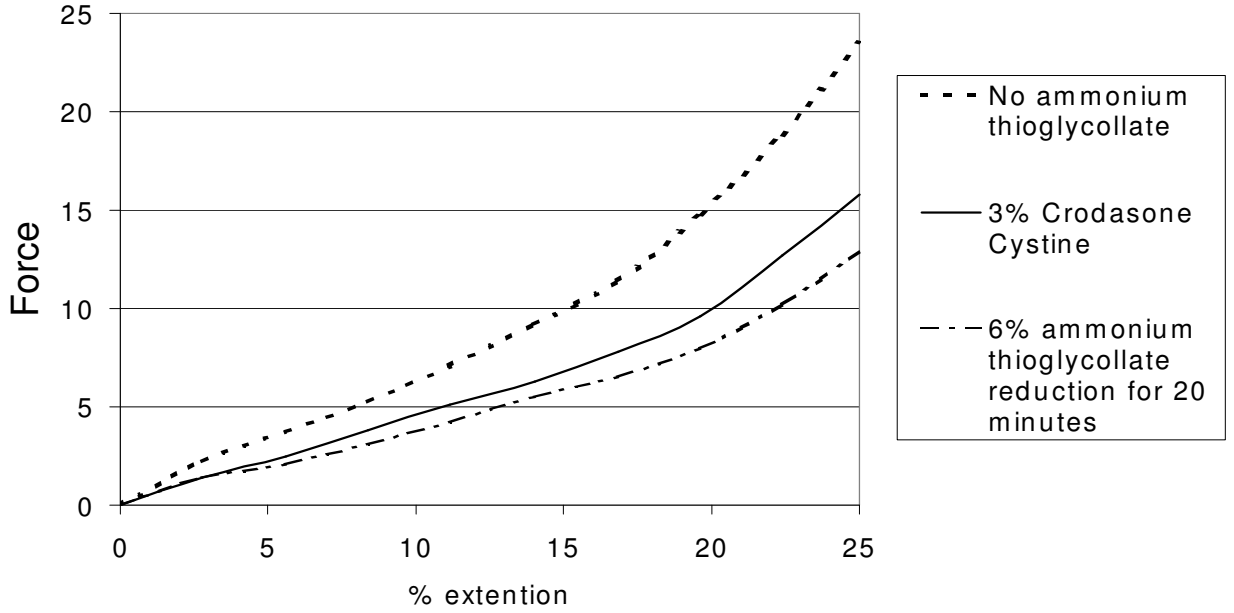




Internal Di-sulphide Exchange

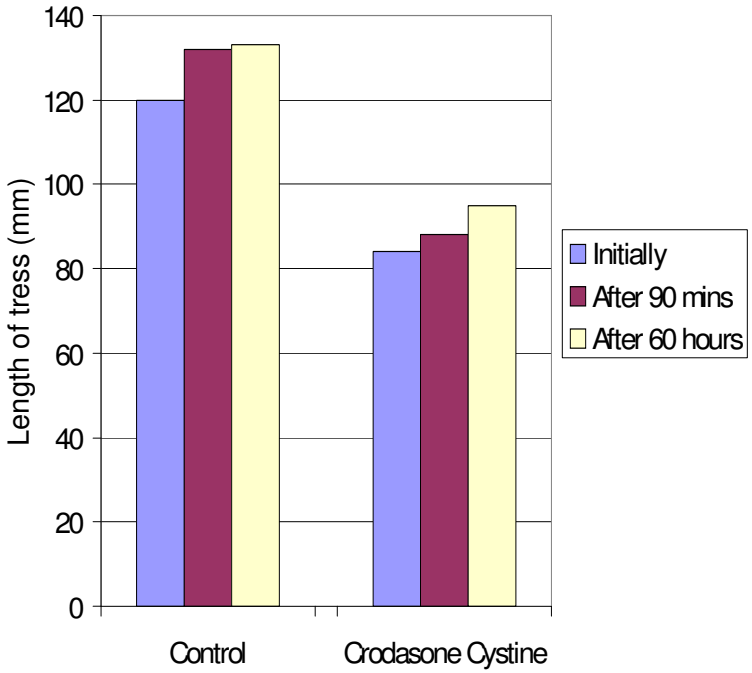
- By measuring force-elongation before and after reduction with ammonium thioglycollate the number of disulphide bonds broken can be determined. On subsequent treatment of the hair fibre with Crodasone Cystine the number of disulphide bonds reformed can be determined.

- Treatment with Crodasone Cystine caused a significant increase in F(25), which was equivalent to approximately 15% of the cystine bonds being reformed. This can be directly attributed to disulphide interchange between thiol groups in the hair and cystine residues in Crodasone Cystine.



Internal Action – Style Aid: Curl Retention

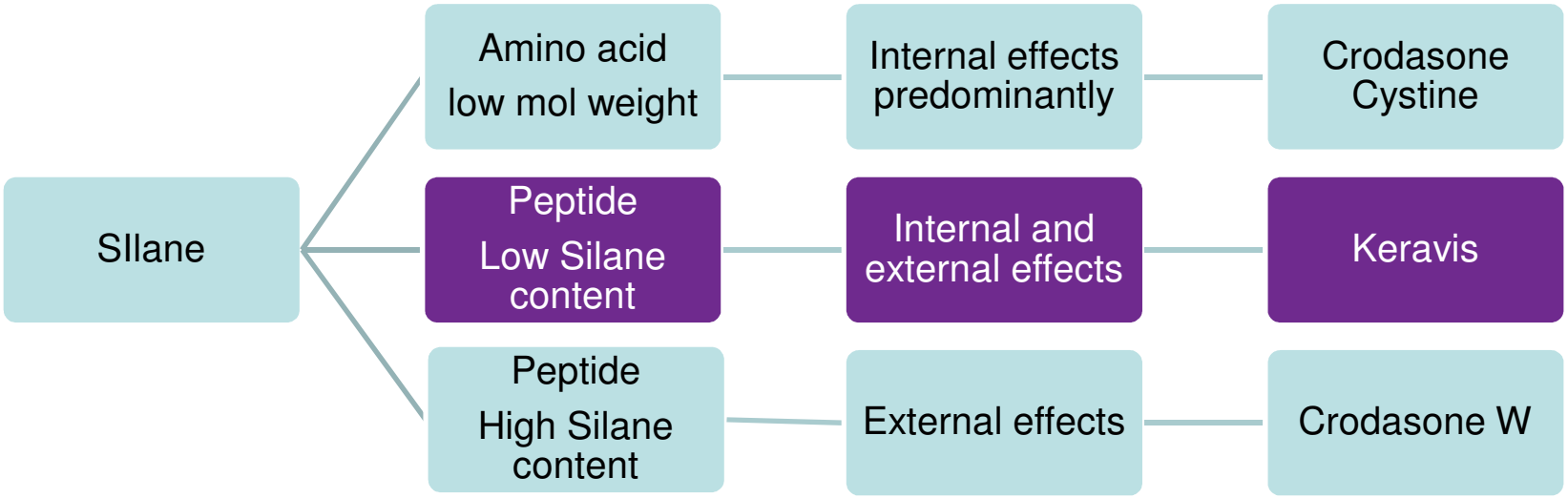
1. Tresses of length 190 mm, was immersed in water
2. The hair was wound onto rollers and immersed in 10% ammonium thioglycollate solution
3. The hair was rinsed in water.
4. The hair was immersed in the test solutions
5. The hair was immersed in 4% hydrogen peroxide
6. The hair was dried at 60-70^{o}C
7. The tresses were hung from a support bar and the length of the curls measured



The results demonstrate that Crodasone Cystine improves the curl retention of hair that has undergone the permanent wave process. This is a clear illustration of the reduction in curl fall-out that can be achieved by directly attaching a molecule capable of cross-linking to the hair shaft.

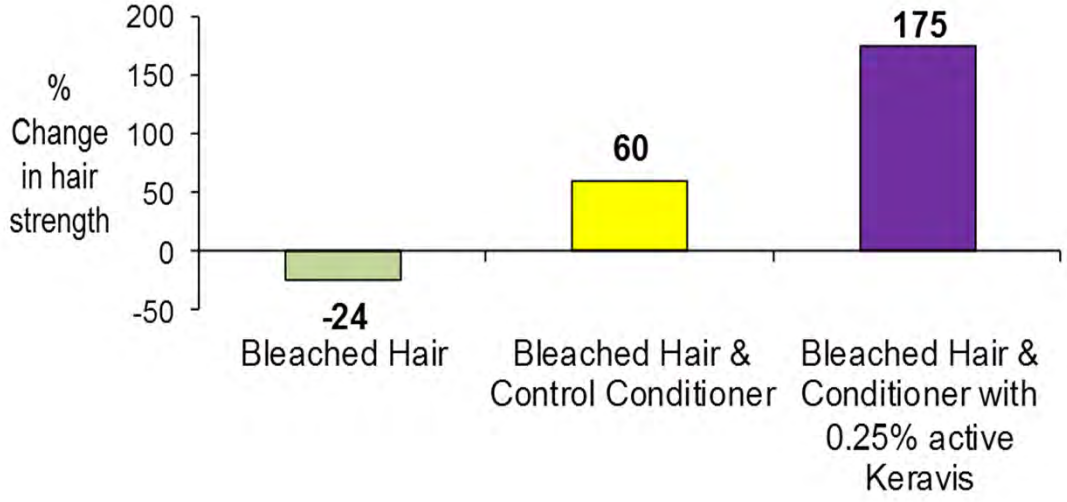
Silane Technologies

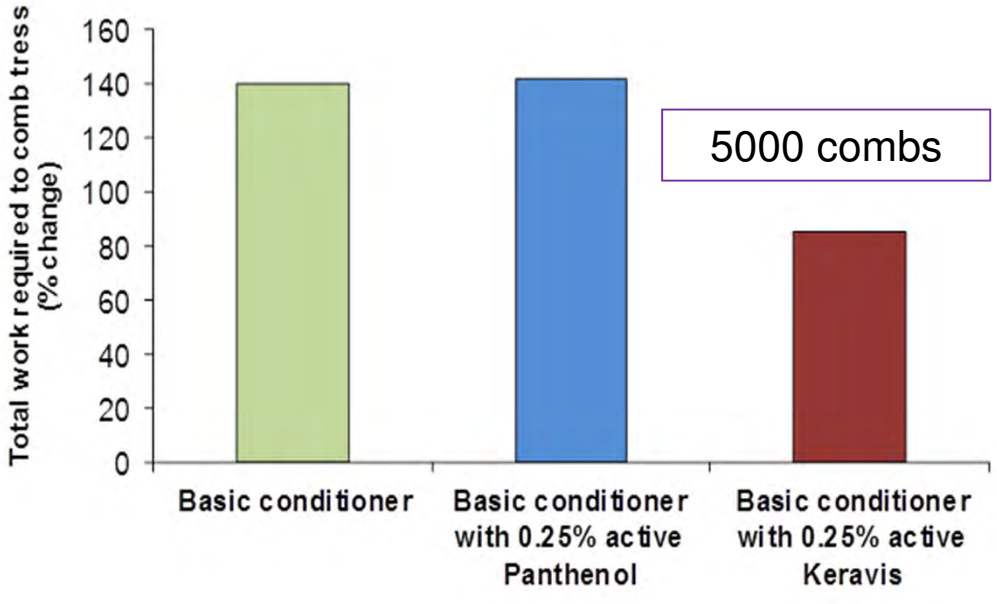
- Applying silanes to different base peptides can impart different end functionality. The effects can be further altered by changing the level of substitution applied



Silanated Peptide – Low % Silane

- The peptide has a low level of silanation giving it the ability to penetrate into the cortex and also be substantive to the surface
- Cortex components make the hair more flexible and resistance to combing forces. They act to mediate the water present to make the hair have an increased tensile strength.
- Surface components cross link to make the hair fibres more resistance to combing abrasion and also reinforce the cuticle to impart further strength





Silane Technologies

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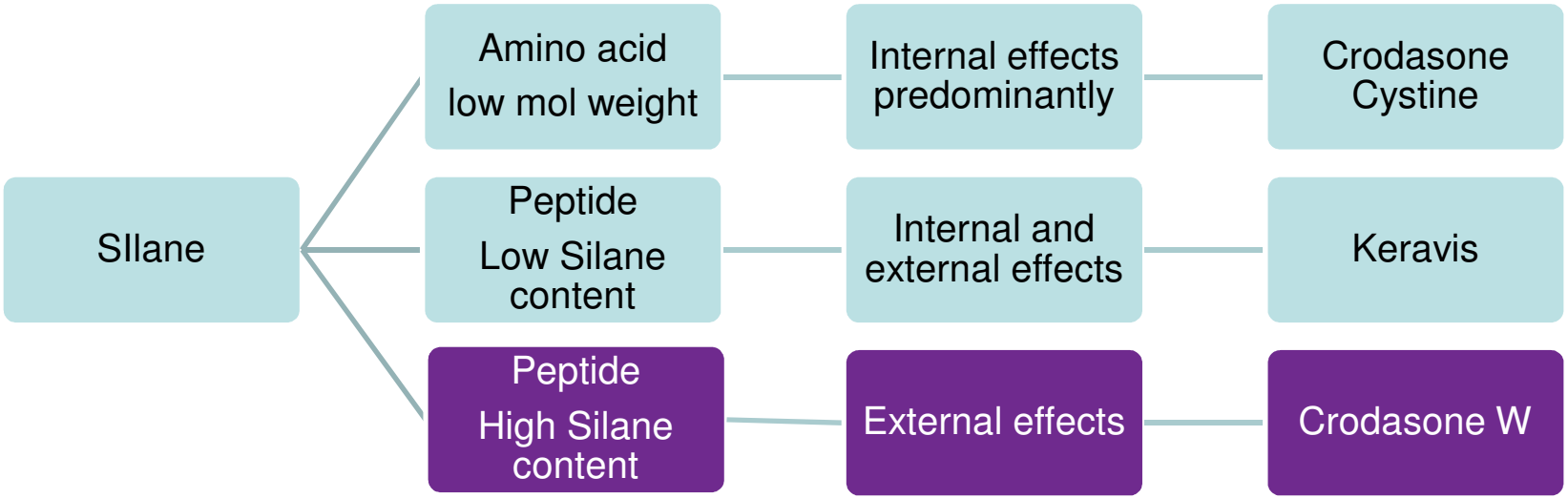
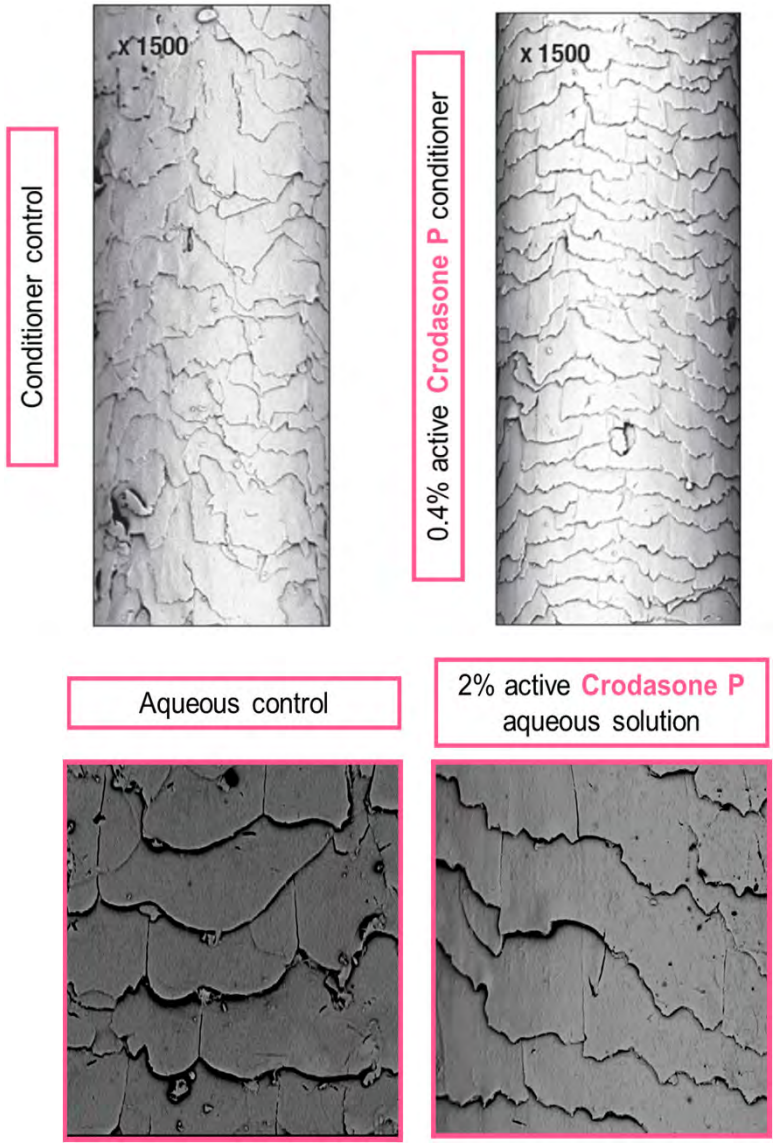


Silanated Peptide – High % Silane

- When the hydration water of the hair is drastically reduced the cuticles crack by circumferential extension stresses arising from the pressure of the swollen cortex.
- A low molecular weight peptide with a high percentage of silane derivative can protect the hair fibre from these stresses
- The product is substantive to the hair fibre and forms a strongly crosslinked network upon drying
- This has been shown to have a protective effect to reduce the numbers of cuticle cracks appearing upon blow drying



Silane Summary

- Silane technology can be very powerful in hair care applications
- The use of the base protein knowledge allows us to pick and choose combinations that can impart beneficial effects to the cortex, cuticle or both
- We can chose the levels and types of silane to attach to the base peptide to further differentiate the products and effects
- Effects of cross linking can be seen as repair and protection of the hair fibre

Personal Care

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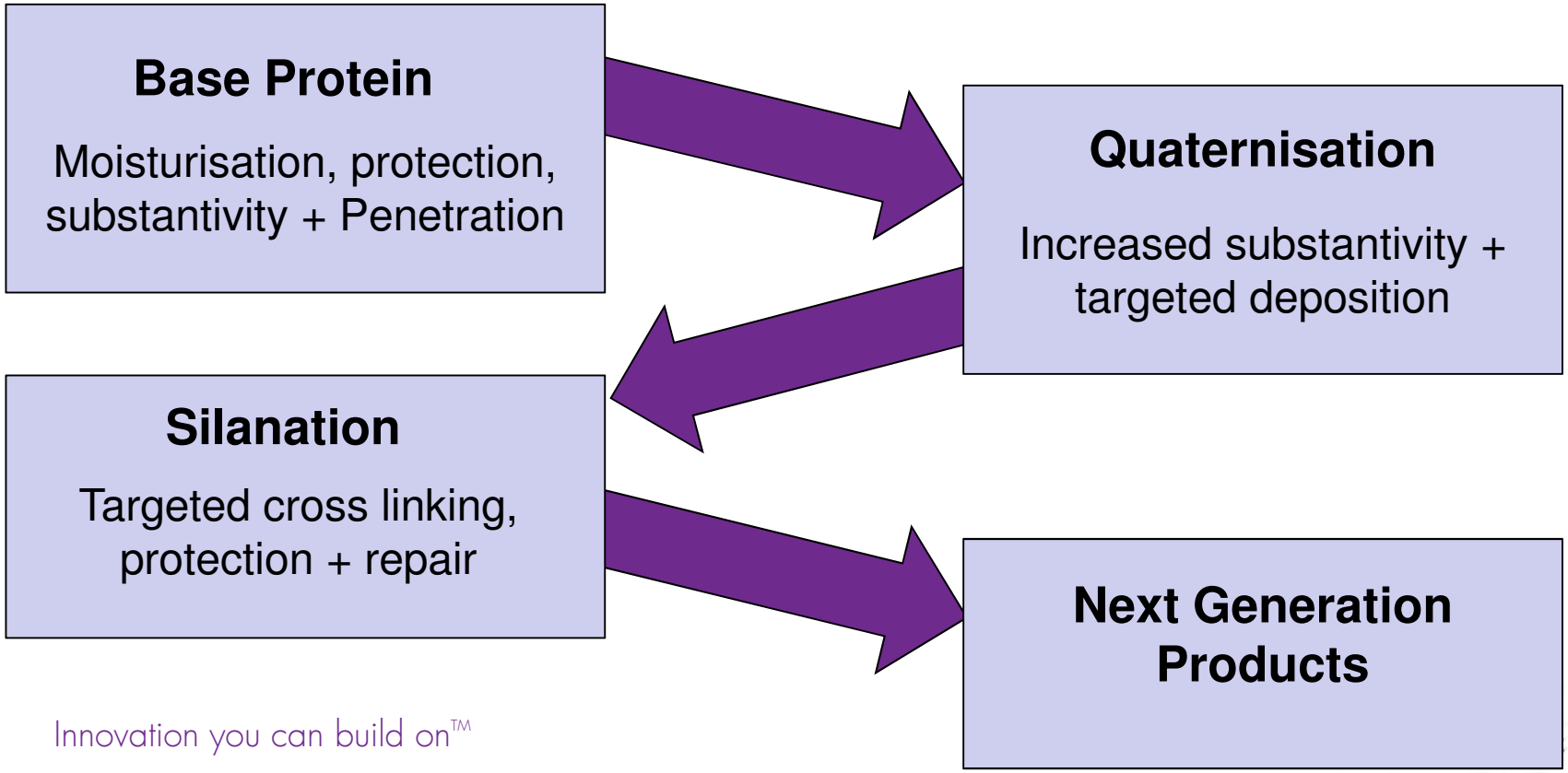
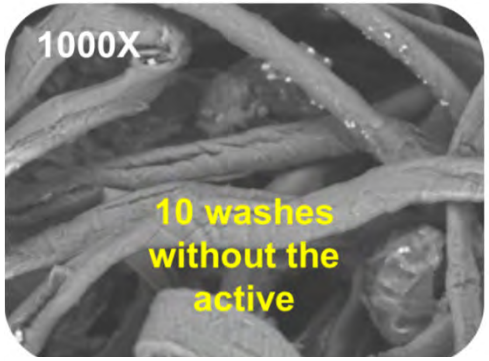
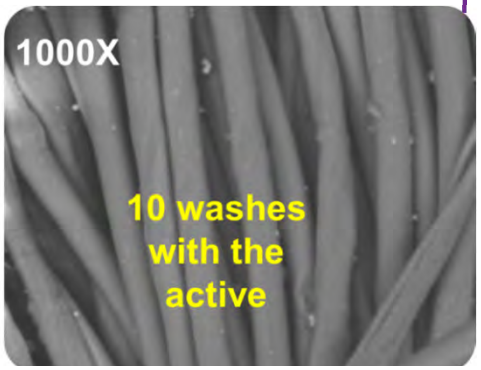
Combining Derivatives

- By taking the knowledge of protein hydrolysis, silanation and quaternisation we can develop further products into other areas of Croda's market place

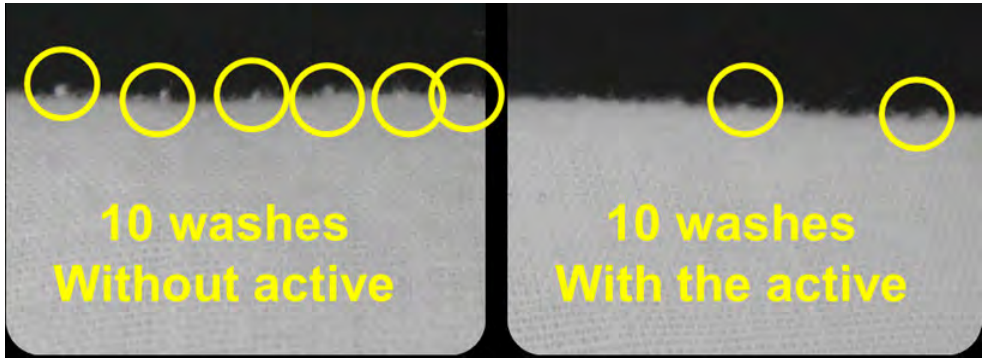


Home Care – Fabric Application

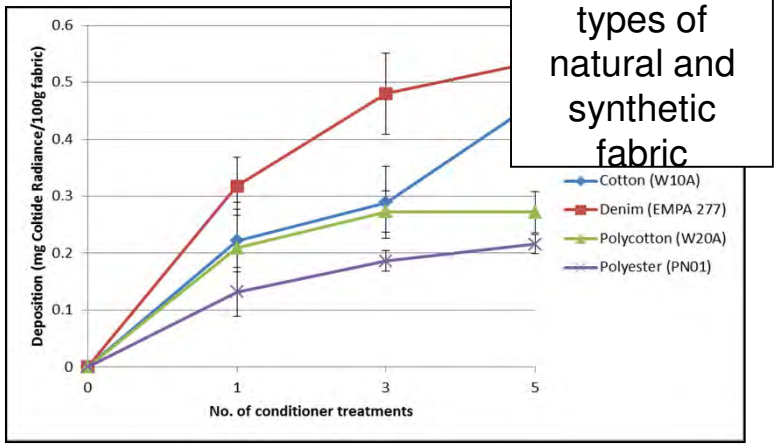


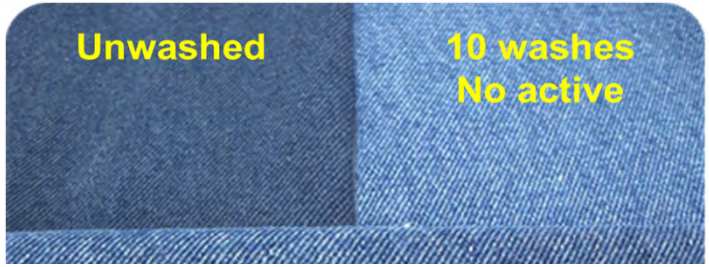


Reduces fibre breaking and fibrillation through moisturisation of the fibres and prevents surface pilling



Binds to all types of natural and synthetic fabric





27% Reduction in colour fade

- We can apply hair care techniques to substantiate claims into home care

Summary

- Proteins are natural molecules showing a huge variety of chemistry. Simple hydrolysis and careful selection of protein source and molecular weight can impart substantiated claims for the hair care market
- Derivatives of proteins can then expand into new products having wide ranging claims and applications ranging from mild surfactants through to home care applications
- For hair care they can be used for emotive label claims (novel sustainable sources), manageability, conditioning, strength protection and repair amongst *many* others.
- Further developments are underway to explore existing technologies further but also investigate new technologies for proteins and protein actives and relating these directly to the chemistry of the hair fibre through proteomics

Personal Care

CRODA

Thank you

Personal Care

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Non-warranty

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