Hair—the most sophisticated biological composite material

Crisan Popescu and Hartwig Höcker*

Received 16th August 2006
First published as an Advance Article on the web 15th February 2007
DOI: 10.1039/b604537p

Hair is a proteinaceous fibre with a strongly hierarchical organization of subunits, from the α-keratin chains, *via* intermediate filaments, to the fibre. The chemistry of the different morphological compartments results in exciting physical properties, including the hydrophilic/hydrophobic paradox. The present *tutorial review* will be of interest for protein- as well as polymer chemists, who want to learn from nature, and also for biochemists interested in the cytoskeleton and particularly in intermediate filaments; it also presents a scientific basis for hair cosmetics.

1. Introduction

Hair is the filamentous appendage of the skin of vertebrates serving to protect the body mainly against coldness and wetness. For human beings, hair has been of particular importance from their earliest times, as a symbol of strength, power, and beauty. Hair has accompanied human development for these mentioned qualities since the dawn of civilisation until today. Animal hair (particularly sheep's hair, *i.e.* wool) is considered to be the first textile fibre used by mankind for insulation and protection; archaeological findings are dated around 5000 BC. Human hair has evolved from a symbol of social status to a main subject of fashion, extensively addressed by an ever-growing cosmetic industry.

In spite of such a glorious history, the investigation of the structure of hair fibres was initiated only about a hundred years ago, and it seems that there are many wonders still to be disclosed.

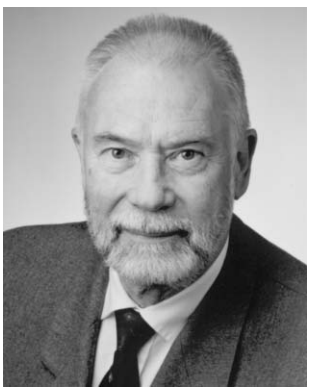
DWI an der RWTH Aachen e.V., Pauwelsstrasse 8, D-52074 Aachen, Germany. E-mail: popescu@dwi.rwth-aachen.de; hoecker@dwi.rwth-aachen.de; Fax: +49 241 8023301; Tel: +49 241 8023300

Hair was shown to be a proteinaceous material and many of the results of hair science research are also cornerstones of protein science. There are two major classes of proteins: the fibrous and the globular ones. Fibrous proteins are distinguished from globular proteins by their filamentous, elongated form and most of them play a structural role in animal cells and tissues. The fibrous proteins comprise, beside the α-keratins in human hair, wool, fingernails, and claws, the fibroin of silk, collagen, the most abundant protein in vertebrates, and actin and myosin in muscles. Beside their form, proteins are also classified as soluble and insoluble, and the fibrous proteins are known to have low solubility. Among them, however, the α-keratins are by far the ones with the lowest solubility.

In the twenties of the last century it was for the first time suggested that the cross-linking by cystine residues, a tetrafunctional amino acid residue of hair comprising a disulfide bond, is the reason for the extreme insolubility of hair. This explanation is still accepted and also allows us to understand the high temperature stability of these fibres.[1] The cystine cross-links were shown to be the reason for the thermal stability of many other proteins, which are of interest as enzymes that are active at elevated temperatures.[2]

Crisan Popescu

Crisan Popescu, was born on July 17, 1953. He studied chemistry at Iasi Polytechnical University, Romania, and Shanghai Textile Institute, China. He received his doctorate in chemistry at the University of Bucharest. In 1995 he began a habilitation at the University of Arad, and became a professor at the university. Since 2001, he has also been a scientist at the German Wool Research Institute at RWTH Aachen Inc. His fields of interest include biomaterials, the chemistry of proteins, thermal analysis, and the kinetics of solid state reactions.



Hartwig Höcker

Hartwig Höcker was born on October 18, 1937. He studied chemistry at Mainz University. In 1965 he received his doctorate in natural sciences. He was a Harkness Fellow of the Commonwealth Fund of New York and a Research Associate at Stanford University, CA, USA, with P. J. Flory. After his habilitation in 1972 at Mainz University he was professor at the Universities of Mainz, Munich and Bayreuth. Between 1985 and 2003 he was a professor at Aachen University and the director of the German Wool Research Institute at RWTH Aachen Inc. He is Editor-in-chief of the electronic journal e-Polymers. His fields of interest include polymer chemistry, biomaterials, and the chemistry of proteins. He is a doctor honoris causa of the University of Arad, Romania.

Also during the twenties, X-ray diffraction was used to investigate the crystalline structure of hair fibres.[3] Astbury *et al*. found two distinct X-ray patterns (α-helix and β-sheet) in the normal and stretched states of the fibre.[4,5] It took twenty more years until Pauling *et al*. came up with a model to give an account of the experimental data.[6]

Over the past several years, however, the hair follicle has achieved increased importance as a system for studying developmental events and the process of terminal differentiation.[7]

Under the microscope, all hair fibres look like monofilaments, covered with scales overlapping like roof tiles which are oriented to the tip of the fibre. In spite of chemical and structural similarities, hair fibres are differentiated according to the mammal source. Forensic science was probably the first to demand the identification of hair origin, and the textile industry, confronted with fakes of cashmere on the market, supported this. The geometry of the scales, and their sequence, give the basis of their identification.[8]

There are other differences between hairs of various sources, for example between the subunits (the cell residues) which form the fibre. Basically, the hair fibre consists of cuticle (outer layer) cells and cortex (core shaft) cells. The cortex may be formed of up to three types of cells, *i.e.* the ortho-, the para-, and the meso-cortex cells.[9] Additionally, in coarse hair there are air-field cavities made up of special types of cells, forming a central medulla. Ortho- and para-cortex cells are found in Merino wool fibres arranged as two strands twisted together and producing the crimp of the fibre, exposing the ortho-cortex cell strand to the exterior and the para-cortex cell strand to the interior of the curl of the fibre. In others wools (coarse wools, such as those from Lincoln sheep) ortho- and para-cortex cells are arranged as concentric cylinders, with ortho-cells always located in the core. Other hair fibres have only one type of cell, usually ortho-cortex cells. The cells differ in terms of their degree of crystallinity, although not in a very clear way: electron diffraction data indicate that ortho-cortex cells have a twofold higher α-helix content than para-cortex cells;[10] on the other hand, from the fusion enthalpy determined by means of differential scanning calorimetry (DSC) there is evidence for lower crystallinity of ortho- than of para-cortex cells.[11] There are also differences in the organisation of the cell components: ortho-cortex cells exert their components, the microfibrils, in a helical array (whorl structure), making the resulting macrofibrils a more distinct entity or more clearly packed than in para-cortex cells which, in turn, exert the microfibrils in an array parallel to the fibre axis.[12]

Looking in more detail, the crystalline domain of hair is formed by a group of α-helices known as intermediate filaments (IFs). These organised proteins are found in nearly all eukaryotic cells forming the cytoskeleton, together with microtubules and microfilaments (see Fig. 1). There are, however, some fundamental differences between the roles played by each of them inside the cell.

The cytoskeleton is a dynamic structure that maintains cell shape, enables some cell motion (using structures such as flagella and cilia), and plays an important role in both intracellular transport (*e.g.*, the movement of vesicles and organelles) and mitosis.

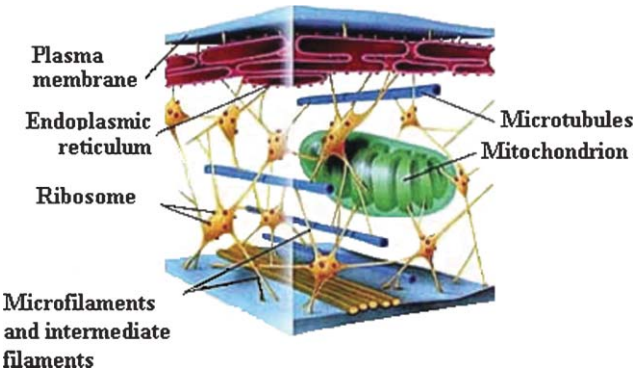


Fig. 1 The structure of a eukaryotic cell. The presence of IFs, microtubules and microfilaments is shown. (Image from Prentice Hall.[49])

The **microtubules**[13] are hollow cylinders of about 24–25 nm diameter, formed by 13 protofilaments which, in turn, are polymers of α- and β-tubulin. They are stiffer than microfilaments and intermediate filaments and play a key role in intracellular transport (associated with dyneins and kinesins they transport organelles such as mitochondria or vesicles), the axoneme of cilia and flagella, the mitotic spindle, and the synthesis of the cell wall in plants.

The **intermediate filaments**[14] are 8 to 11 nm in diameter, and are the most stable (strongly bound) and heterogeneous constituents of the cytoskeleton. They organize the internal three-dimensional structure of the cell (*e.g.*, they are structural components of the nuclear envelope or the sarcomeres). They also participate in some cell–cell and cell–matrix junctions.

Different intermediate filaments are made of various subunits belonging to one of the following six classes.

Type I and type II IFs are acidic and basic α-keratins, respectively. They associate in a 1 : 1 ratio to form heterodimers, which assemble into microfilaments. Neither type can assemble on its own.

About 10 keratins are specific for the hard epithelial tissues (hair, nails, and wool) and about 20, the cytokeratins, are more generally found in the epithelial cells which line body cavities. Each type of epithelium expresses a characteristic combination of type I and type II keratins.

Four proteins are classified as type III IFs. These proteins can form both homo- and heteropolymers. The most widely distributed protein of this type is vimentin, which is expressed in leukocytes, blood vessel endothelial cells, some epithelial and mesenchymal cells such as fibroblasts. Vimentin is often associated with microtubules and is considered to keep the nucleus and other organelles in place. Desmin is found in muscles and is responsible for stabilizing the sarcomere. Glial fibrillary acidic proteins form filaments in glial cells that surround neurons. Peripherin is found in neurons of the peripheral nervous system.

The type IV IFs form neurofilaments. Neurofilaments (NFs) in mature neurons are heteropolymers of NF-L, NF-M, and NF-H that differ greatly in molecular weight. NFs are responsible for the radial growth of axons and determine the axon diameter, which is related to the velocity of impulse conductance. In quails there is a mutation that affects the NF

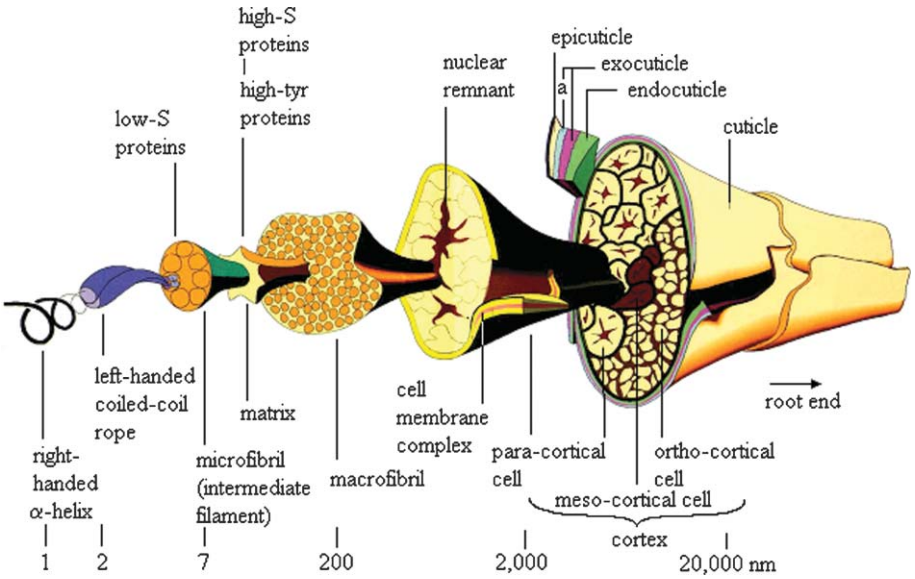


Fig. 2 Structure of the α-keratin fibre under various magnifications.[16] (Reproduced with permission from John Wiley & Sons, Inc.)

assembly and the velocity of nerve conduction is severely reduced.

The non-standard type IV filaments are found in the eye and have no similarity to other IFs.

The type V IF proteins, the laminins, are found in the nucleus. Of the three nuclear laminins, two are alternatively spliced products encoded by a common gene, while the third one is encoded by a separate gene.

The various types of IF proteins are associated with diagnosing and treating certain tumours. Whether a tumour originates in epithelial, mesenchymal (connective tissue, blood vessels, lymphatics), or neuronal tissue can be determined using fluorescently-labelled antibodies against the respective IFs. The most common tumours come from breast and gastrointestinal epithelia and contain the respective α-keratins.

The **microfilaments**[13] (actin filaments) are the thinnest component of the cytoskeleton, measuring 7 nm in diameter. They are formed by the polymerisation of G-actin, a globular protein, into a helical shape to build the microfilament called F-actin.

Beside the mechanical support for the cell provided by actin filaments, they also determine the cell shape and enable cell movement (through lamellipodia, filopodia, or pseudopodia). Actin filaments are essential components of the muscle cells and are responsible, along with myosin, for muscle contraction. Actin is one of the most abundant proteins in many eukaryotic cells, with concentrations of over 100 μM. It is also one of the most highly conserved proteins, differing by no more than 5% in species as diverse as algae and humans.

2. Morphology of hair

The protein fibre hair is a multicellular tissue of several morphological components, each with a specific chemical composition.[15] The fibre geometry is roughly cylindrical, with a diameter ranging from 10 μm for fine hairs (Angora rabbit, Cashmere goat) to more than 100 μm for the coarse fibres of yak, sheep or human hair. When including the spines of the porcupine or the bristles of the manes of lions among hairs, the diameter goes to even larger values.

Generally speaking, a hair fibre is composed of the cortex and the cuticle. Each of the two components is formed of various other morphological components. The cortex contains cortical cells and the cell membrane complex. The cortical cell is further composed of macrofibrils and intermacrofibrillar material. The macrofibrils consist of microfibrils and an intermicrofibrillar matrix. In summary, the cortex is formed of microfibrils (IF or keratin proteins KP) and IF- or keratin associated proteins (IFAP or KAP), which compose the intermicrofibrillar matrix containing cytoplasmatic and nuclear remnants. This ensemble is wrapped up in the cuticle, as an external sheath which has also its own architecture, being formed of four layers: the epicuticle, the a-layer, the exocuticle and the endocuticle (Fig. 2). Thus, the α-keratin fibre is the best example of a natural composite system, having a complex dual structure at all levels (Table 1).

The cuticle consists of plate-shaped cells (''scales'') that overlap longitudinally and peripherally, with up to 1 μm thick edges of the scales pointing to the tip of the fibre. Each cuticle cell consists of four layers with different content of disulfide

Table 1 The composite structure of the hair fibre

Composite	Type	Component 1	Component 2
Hair fibre	Ring/core	Cuticle	Cortex
Cortex	Filament in matrix	Cortex cells (spindle shape)	Cell membrane complex
Cortex cell	Filament in matrix	5–8 Macrofibrils	Intermacrofibrillar matrix
Macrofibril	Filament in matrix	500–800 Microfibrils (IFs)	Intermicrofibrillar matrix

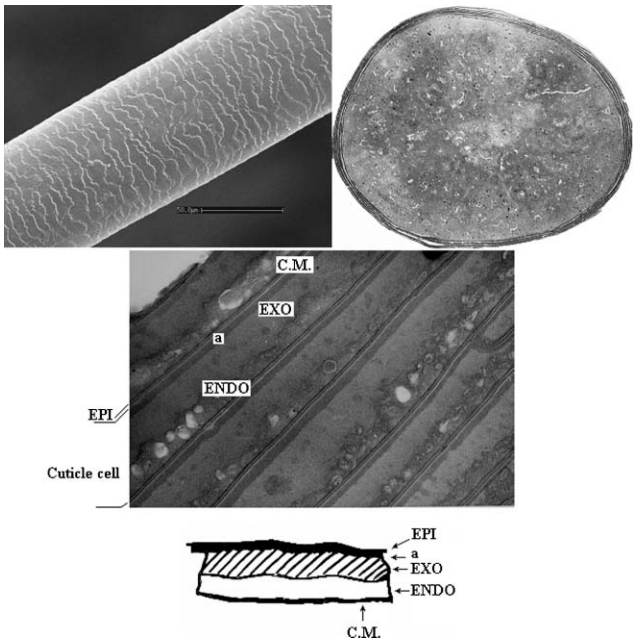


Fig. 3 Scanning electron micrograph showing the hair fibre shaft and its cross-section (above) and transmission electron micrograph of the hair cuticle (middle). The schema (below) shows the layered structure of the cuticle. EPI, epicuticle; a, a-layer; EXO, exocuticle; ENDO, endocuticle; C.M., cell membrane.

and isodipeptide bonds (Fig. 3): the epicuticle at the very surface, which is an approximately 5 nm thin hydrophobic resistant membrane; the a-layer; the exocuticle; and the endocuticle. The cell membrane complex binds overlapping cuticle cells together.[17]

The cortex consists of spindle-shaped cells. Ortho-, para-, and the more rare meso-cortical cells differ in their cystine content and staining behavior. A cortical cell contains around 5–8 macrofibrils with a diameter of 300 nm at their widest point.

Cytoplasmic and nuclear remnants of the keratinocytes make up the intermacrofibrillar matrix. A macrofibril is a bundle of 500–800 microfibrils.

Keratin associated proteins (KAP) make up the intermicrofibrillar matrix. Keratin microfibrils have a unique lateral organization.[18] The number of keratin chains in the cross-section is about 32, which, within the limits of experimental error, is the value obtained for intermediate filaments from other sources as well.

The microfibrils consist of 7 or 8 single or paired protofilaments. The subunit of the protofilament is the four chain structure discovered by Ahmadi and Speakman.[19] 7 or 8 protofilaments contain, therefore, 28 or 32 keratin chains, in good agreement with the data of Jones and Pope.[20] The arrangement of chains into protofilaments and protofibrils is shown in Fig. 4.

The overall morphology, at various scales, of a hair fibre is given in Fig. 5.[21]

The medulla in coarser hair fibres consists of hollow cells with a skeleton of amorphous proteins and fine filaments.

The complex morphological and molecular structure of a hair echoes the construction principle of all biological

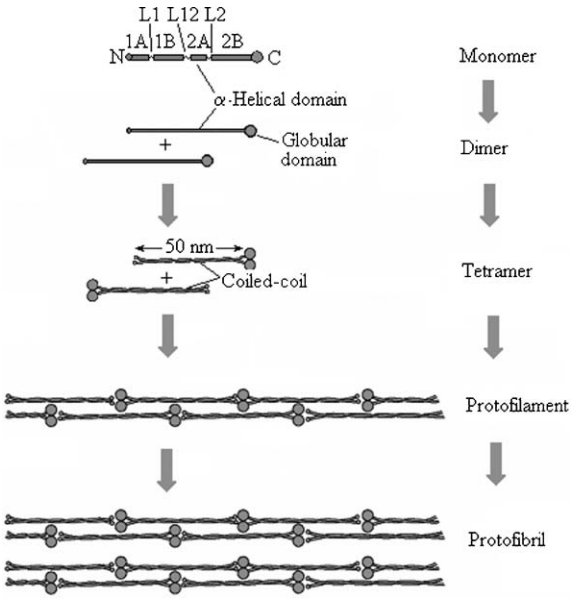


Fig. 4 Structure of the intermediate filaments. A and B: helical domains; L: non-helical linkers; N, C: N and C termini, respectively.

composite structures (further examples are the hierarchical structure of collagen, cotton fibres and chromosomes) and many man-made materials (*e.g.* carbon fibre reinforced epoxy resins), combining components with different properties in one material so as to maximize suitability for its purpose.

The natural colour of hair is caused by pigment granules of black to brown eumelanin and yellow to red pheomelanin. Melanin pigments also exist in skin cells, being responsible for body protection by absorbing UV radiation; neuromelanin forms the *substantia nigra* in the brain and there are observations showing that Parkinson's disease is related to the disappearance of this dark matter.[22]

The melanin pigments are conjugated polymers[23] synthesised in the body of mammals from tyrosine, which is transformed into dopaquinone and, following different pathways (which may include reaction with cysteine or leucodopachrome), produces benzothiazines and 5,6- dihydroxyindol, the monomers which polymerise to produce pheo- and eumelanin.[24] The melanin production cycle in the melanocyte

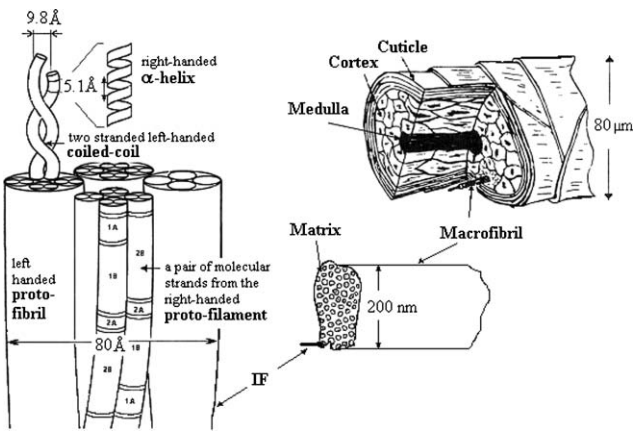


Fig. 5 The morphology of a hair fibre.

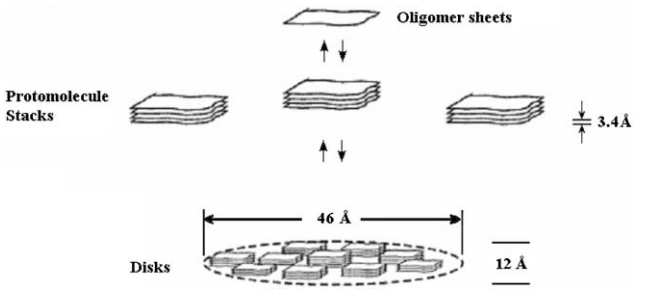


Fig. 6 The morphology of a melanin granule.[25]

cells of hair follicles may stop due to various reasons (anaemia, thyroid, age) leading to grey (partially unpigmented), or even white (totally unpigmented) hair.

The melanin granule is also a structured body in which sheets of oligomers stack to form 5 nm disk-like particles that aggregate into spheres of 70–500 nm diameter (Fig. 6).[25]

The size of the granule is related to the absorbed radiation and, therefore, to the colour of the fibre. Thus, the colour of hair fibres is not based on chromophore chemistry but on geometry (physical colour).

It is the destroying of these granules that leads to hair bleaching. The process is imagined as a de-layering of the discs of melanin under the influence of hydrogen peroxide, followed by the elimination of the oligomeric sheets. The granules become smaller, physically inducing the modification of colour from dark to blond shades.[25] The sequence suggests that hydrogen peroxide interacts preferably with the melanin discs, and less with the keratin. This explains why bleaching of a pigmented fibre damages the fibre less than would be expected from the reaction of keratin with hydrogen peroxide.[26]

An activated (catalysed) oxidation of the melanin pigment is used for selective bleaching of pigmented fibres in wool, or cashmere products. The process is based on the fact that Fe^{2+} selectively complexes with melanin disks[27] and on the catalytic effect of Fe^{2+} on the decomposition of H_2O_2.

3. Chemical composition of morphological compartments

Hair fibres are hygroscopic, and the amount of water taken up depends on the relative humidity of the air, temperature, and the history of the fibre. The sorption and desorption curves exhibit a hysteresis, reflecting the different behaviour of the fibre on up-taking compared to releasing water molecules (Fig. 7). At average relative humidity, the difference between the two curves is about 2%. The moisture uptake is accompanied by a dissipation of heat which achieves around 110 J g^{-1} for the sorption of 18% moisture.[28]

Although hair fibres are hygroscopic, their surface is hydrophobic towards liquid water. This apparently contradictory behaviour, the "hair paradox", which is an important factor for the physiological effects of wool as a clothing material, is due to the fact that the interior of the fibre contains hydrophilic amino acid residues, whereas the very surface of the cuticle presents the aliphatic chains of fatty acid residues. The low surface tension of undamaged hair (of about 30 mN m^{-1}) is easily increased following *e.g.* oxidative

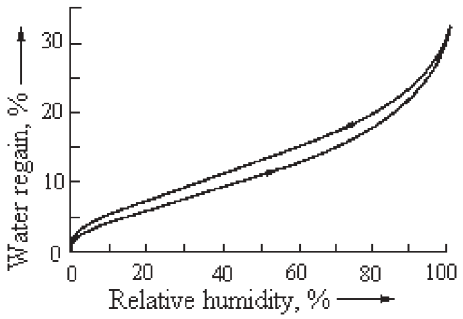


Fig. 7 A typical sorption–desorption hysteresis recorded for a wool fibre over the range of relative humidity from 0 to 100% at 25 °C.

treatments, which explains the easier wetting of a bleached hair compared to an untreated one. The hydrophobic nature of the surface also plays an important role when designing coating formulations for imparting lustre to hair fibres.

Water absorption results in swelling, the hair fibre showing considerable swelling anisotropy (fibre swells differently along length and radius directions) (Fig. 8). An increase in the amount of absorbed moisture from 0% to 33% leads to a longitudinal swelling of *ca.* 2% and a radial swelling of 16%. The extent of swelling depends on the pH value and the swelling medium.

The degree of fibre swelling is smallest at the isoionic point, *i.e.* at pH 5.5. Above and below this point, the number of stabilizing salt bridges decreases and the peptide chains carry excess negative or positive charges which cause electrostatic repulsion, resulting in increased swelling. Water is, therefore, a

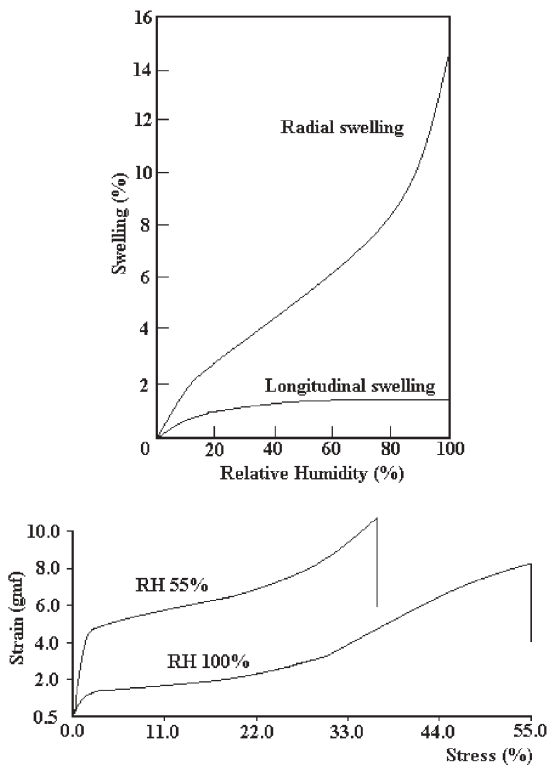


Fig. 8 Effect of moisture sorption on swelling (top) and elasticity (bottom) of a hair fibre.

main component of hair fibres, and plays an important role in its mechanic performance.[29]

The decrease of the elasticity modulus, shown also in Fig. 8 (the stress–strain diagram), with increasing water regain explains why hair looks flabby when in an environment of high relative humidity.

The large radial swelling, with up to 16%, and the swelling in length of *ca*. 2%, leads to a quite significant outer surface extension of about 20% and, as a consequence, to an increase of the diameter of the fibre pores by about 10%. This helps to understand how swelling of the fibre assists the diffusion of dye molecules, following the pore model of the theory of dyeing.[30]

Water-free hair fibres consist mainly (90–97%) of proteins and 2% of lipids, the remainder being made up of nucleic acids, carbohydrates and inorganic substances.

The elemental analysis of hair shows around 50 wt% carbon, 7 wt% hydrogen, 22 wt% oxygen, 16 wt% nitrogen, and 5 wt% sulfur. The percentages differ slightly with the source of the hair, but keep a remarkable constancy around the mentioned values. The high sulfur percentage results from the high cystine content. This is the characteristic feature of α-keratin fibres and distinguishes them from other protein fibres such as silk and collagen.

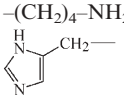
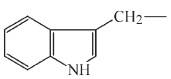
Total hydrolysis of the peptide bonds in hair proteins yields the 20 common natural α-amino acids and ammonia and, in addition, small amounts of thiocysteine, cysteic acid and lanthionine. The data given in Table 2 were obtained by combining data from acidic and from enzymatic hydrolyses of wool, as an example of an α-keratin fibre.[31]

The amino acids are classified into five groups: ''acidic'' amino acids, ''basic'' amino acids, amino acids with hydroxyl groups, sulfur-containing amino acids, and amino acids with no reactive groups in the side chain. The total amount of amino acids with reactive side groups is 4940 μmol g^{-1}, and of amino acids without reactive side groups 3450 μmol g^{-1}. The sum of these two figures, after subtraction of the concentration of C-terminal amino acids (10 μmol g^{-1}), results in 8380 μmol g^{-1} for the concentration of peptide groups in the peptide chains of hair.

Like all proteins, hairs contain both cationic and anionic groups, and are therefore amphoteric. The cationic character is due to the protonated side groups of arginine, lysine, and histidine, and free terminal amino groups. The thiol groups of cysteine and the amino groups of lysine, histidine, and the amino end groups are important sites for the covalent (electrophilic) attachment of chemical reagents and reactive dyes. Anionic groups are present as dissociated side groups of aspartic and glutamic acid residues and as carboxyl end groups.

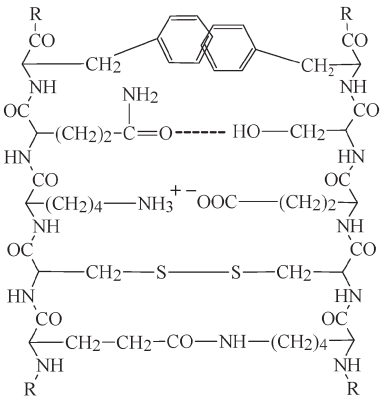
Eight N-terminal amino acids have been determined by means of reaction with 2,4-dinitrofluorobenzene (Sanger reagent): cystine, glycine, threonine, valine, alanine, serine, and glutamic and aspartic acids. Other N-terminal amino acids

Table 2 Amino acid composition of Merino wool[31]

Group	Name	Side chain	Concentration/μmol g^{-1}
''Acidic'' amino acids and their ω-amides	Aspartic acid	$-CH_2-COOH$	200
	Glutamic acid	$-(CH_2)_2-COOH$	600
	Asparagine	$-CH_2-CONH_2$	360
	Glutamine	$-(CH_2)_2-CONH_2$	450
''Basic'' amino acids and tryptophan	Arginine	$-(CH_2)_3-NH-C(NH_2)=NH$	600
	Lysine	$-(CH_2)_4-NH_2$	250
	Histidine		80
	Tryptophan		40
Amino acids with hydroxyl groups in the side chain	Serine	$-CH_2-OH$	900
	Threonine	$-CH(CH_2)-OH$	570
	Tyrosine	$-CH_2-C_6H_4-OH$	350
Sulfur-containing amino acids	Cysteine	$-CH_2-SH$	10
	Thiocysteine	$-CH_2-S-SH$	5
	Cysteic acid	$-CH_2-SO_3H$	10
	Cystine	$-CH_2-S-S-CH_2-$	460
	Lanthionine	$-CH_2-S-CH_2-$	5
	Methionine	$-(CH_2)_2-S-CH_3$	50
Amino acids without reactive groups in the side chain	Glycine	$-H$	760
	Alanine	$-CH_3$	470
	Valine	$-CH(CH_3)_2$	490
	Proline		520
	Leucine	$-CH_2-CH(CH_2)_2$	680
	Isoleucine	$-CH(CH_2)-CH_2-CH_3$	270
	Phenylalanine	$-CH_2-C_6H_5$	260

are present in the N-acetylated form. Thus, the acid hydrolysis of wool liberates 50 μmol g^{-1} of acetic acid.

The side groups, which account for a considerable portion (50 wt%) of the protein molecular mass, interact with each other, thereby stabilising the peptide by forming links between the chains and rings within a chain, as demonstrated by the following schematic representation of five such links between segments of two hypothetical peptide chains.



From the top downwards it shows interactions between phenyl rings,[32] hydrogen bonds between an asparagine residue and a serine residue, a salt bridge between an arginine residue and a glutamic acid residue, a disulfide bridge between two cysteine residues, and an isodipeptide bridge between a glutamic acid and a lysine residue.

The disulfide bond plays an important part in stabilising the hair fibre, leading, in particular, to its relatively high wet strength, insolubility and thermal stability.

A second covalent bridge is caused by the isodipeptide N_{ε}-(γ-glutamyl)lysine residue which provides an additional stabilising effect in the resistant cell membranes and the cuticle.

The disulfide bond, too, is the key for the perm waving of human hair or the setting of wool fabrics. These processes are based on breaking and reforming S–S bonds in new positions after having arranged the hair fibres or the wool fabric in the desired macroscopic shape.[33] Consequently, the treatment is a two-step chemical process combined with a one-step mechanical action, as follows:

1. *Breaking of the disulfide bonds*. This is achieved by treating hairs and wool (W–S–S–W) with an excess of a reducing agent (for cosmetic treatment: thioglycolic acid (TGA) or its ammonium salt; for wool treatment: sodium bisulfite, or an organic bisulfite):

$$\mathrm{W{-}S{-}S{-}W + 2HSCH_2COO^- \rightarrow}$$
$$\mathrm{2W{-}SH + {}^-OOCCH_2{-}S{-}S{-}CH_2COO^-}$$

2. *Shaping the fibres*. After the breaking of the disulfide bonds, the keratin fibre becomes plastic and it is shaped into a desired form. In a cosmetic salon this is done by rolling the hair on curlers; in the textile industry it is performed by pressing the wool fabric.

3. *Reforming the disulfide bonds*. Once the desired shape is achieved, it is ''frozen'' by reforming disulfide bonds with an excess of an oxidising agent. The most widely-used oxidising agent is H_2O_2:

$$\mathrm{2W{-}SH + H_2O_2 \rightarrow W{-}S{-}S{-}W + 2H_2O}$$

Of course, beside the main reaction of cystine reduction there are several side reactions which lead to the formation of cystine degradation products and, consequently, to the loss of cystine available for reformation. Poor control of pH or temperature may favour the side reactions and may lead to a weakening of the fibre strength.

Several other reagents are available for the first step, the breaking of the sulfur bonds, aimed at reducing the damage of hair. The very first perm-wave process, proposed in 1909 by Nessler,[34] used alkaline medium and high temperature to achieve the curling, and it is only since the late thirties of the last century, after Willatt patented his ''cold waving'' system, that reducing reagents have been successfully applied.[35]

The observation that keratin fibres become soft under the effect of a reducing agent, made by Speakman's group[36,37] led not only to the improved perm-waving treatment, but also to a new industrial process for producing permanently stretched (''slenderised'') wool fibres (OPTIM™ technology).[38] The process uses a stretching action for the mechanical step and the fibres are then stabilised in the new elongated form, which makes them thinner and more lustrous. Since the chemical properties of the stretched fibres are also partly changed, the fibres are branded as new, different from wool (as the fibre Arcana™ of The Woolmark Co.).

The analysis of proteins in hair is performed by means of SDS polyacrylamide gel electrophoresis (SDS PAGE). This type of electrophoresis uses sodium dodecyl sulfate (SDS) to mask the protein charge, so that proteins are separated mainly according to their molecular weight. The basic steps of the analysis are:

1. *Reduction and extraction*.

$$\mathrm{{-}S{-}S{-} + dithiothreitol\ or\ dithioerythritol \rightarrow}$$
$$\mathrm{2\ {-}SH + \overline{S{-}CH_2{-}CHOH{-}CHOH{-}CH_2{-}S}}$$

2. *Blocking of free SH-groups*.

$$\mathrm{{-}SH + I{-}CH_2{-}CO{-}NH_2 \rightarrow {-}S{-}CH_2{-}CO{-}NH_2 + HI\ or}$$
$$\mathrm{{-}SH + I{-}CH_2{-}CO{-}O{-}R \rightarrow {-}S{-}CH_2{-}CO{-}O{-}R + HI}$$

3. *Separation of proteins*.

4. *Visualization and staining with a dyestuff, Coomassie blue*.

The analysis produces a partition of the type shown in Fig. 9. The proteins are further analysed chemically and classified as:

Low-sulfur proteins (LS-proteins), *ca*. 50 wt% of the total protein, are considered to be the intermediate filament-proteins. They are partly crystalline, and presumed to form the α-helical components.

High-sulfur proteins (HS-proteins), *ca*. 25 wt% of the total protein, are considered to constitute the intermediate filament-associated proteins (IFAPs), the amorphous part of the fibre.

High glycine and tyrosine proteins (HGT-proteins), *ca*. 10 wt%, are also part of the amorphous IFAPs.

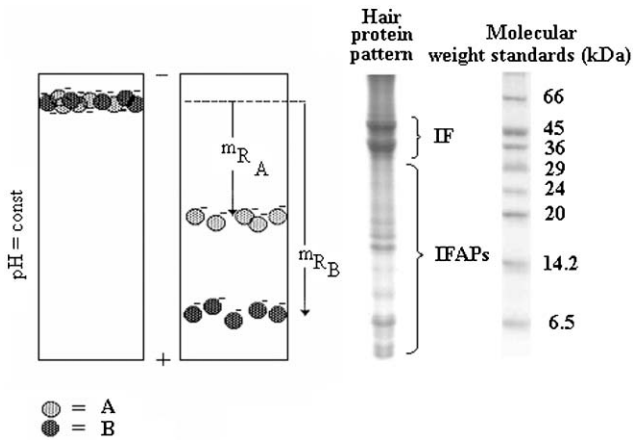


Fig. 9 SDS-Gel electrophoresis (SDS-GE) of hair proteins. The principle of separation due to differences of molecular weight is illustrated at the left, with two different molecules A and B. A typical SDS-GE result of hair proteins after reduction (mercaptolysis) of the disulfide bonds and staining is shown, compared with a standard molecular weight ladder.

Others, low-sulfur and high-sulfur proteins, *ca.* 15 wt% of the total proteins, are amorphous and considered to stem from the exo- and endocuticle, the cell membrane complex and residues of the cells.

While the amino acid composition shows a large degree of similarity when going from one mammalian hair to another, the compositions of the morphological components of cortex and cuticle of the same hair are highly differentiated (Table 3, human hair as an example of an α-keratin fibre).

As is easily noticeable, there are differences not only between the amounts of amino acids in the cuticle and cortex of the same fibre, but also between the amounts of amino acids given in Tables 2 and 3. While the differences between cuticle and cortex reflect differences between the structure and functionality of the two components of the same fibre, the differences between wool (Table 2) and human hair (Table 3) reflect, practically, the different history of the two keratin fibres.

Table 3 Amino acid composition of whole human hair, and of hair cortex and cuticle (in μmol g^{-1})[39]

Amino acid	Whole hair	Cortex	Cuticle
Cysteic acid	32	27	59
Aspartic acid and asparagine	399	416	300
Threonine	554	580	412
Serine	967	850	1628
Glutamic acid and glutamine	916	930	848
Proline	588	532	900
Glycine	437	368	836
Alanine	347	370	500
Valine	405	374	644
Half-cystine	1435	1350	1880
Methionine	13	9	39
Isoleucine	174	172	186
Leucine	457	466	404
Tyrosine	158	162	134
Phenylalanine	124	126	115
Ornithine	—	—	—
Lysine	196	172	331
Histidine	62	65	53
Arginine	466	496	289

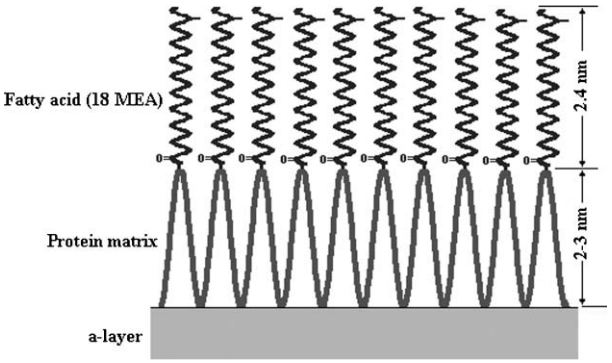


Fig. 10 The surface of the epicuticle with the proposed arrangement of 18-methyl-eicosanoic acid (sketched according to the description in ref. 40).

The cuticle is usually highly abundant in cystine residues, while the cortex has a lower content of this amino acid residue. Beside the highly cross-linked structure, the cuticle contains a low but important amount of fatty acids, in particular of 18-methyl-eicosanoic acid (18-MEA), which is responsible for the water repellent behaviour of the surface. As schematically shown in Fig. 10, the 18-MEA is covalently bound to the a-layer at the surface of the epicuticle, the 2.4 nm long hydrocarbon tail being oriented to the outside.[40]

The peptide arrangement in hair fibre has been the topic of many investigations during the first half of the 20th century. Astbury *et al.*[4,5] used X-rays to demonstrate the nature of a crystalline phase in hair. The X-ray diffraction pattern (Fig. 11) shows a meridian reflection at 0.51 nm and an equatorial reflection at 0.98 nm. Interpreting these results Pauling *et al.*[6] proposed the α-helical structure to give account of the secondary structure of hair, given in Fig. 12.

The α-helix contains 18 amino acid residues in five turns, *i.e.* 3.6 amino acid residues per turn. To result in the distance between successive turns of the helix that leads to the observed meridian reflection (0.51 nm), the helical chain must itself be slightly coiled (super-helix, coiled coil[41]). Two super-helices combine to form a left-handed two-stranded rope-like assembly in which the super-helices are arranged in such a way that the hydrophobic side groups at the outside of the helices interlink to form a stable ''buttonhole'' structure.[42] These dimers are the actual structural subunits of the microfibrils, and can be termed ''molecular twins''.

The force which keeps two α-helices together in the coiled-coil dimer, the first ''brick'' of the IF rod, is the geometry of

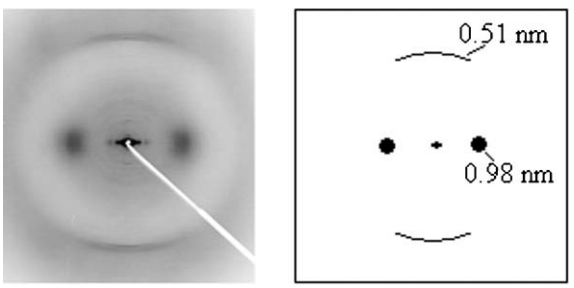


Fig. 11 X-Ray diffraction pattern for an α-keratin fibre and the simplified scheme with the most evident data.

Fig. 12 The arrangement of amino acid residues into the α-helical conformation, *e.g.* of an α-keratin proposed to meet the X-ray diffraction pattern shown in Fig. 11.[6]

the arrangement of amino acid residues in the polypeptide chain and the hydrophobic effect. The geometry requires a repeating sequence of seven amino acids (**abcdefg**), a heptad, with the residues **a** and **d** representing hydrophobic ones, as shown in Fig. 13.[31]

The letters may be replaced by any amino acid residue from Table 2, with the only requirement that amino acids **a** and **d** are hydrophobic ones.[31]

In addition to the organic constituents, trace elements were detected. The ash content of a hair ranges from 0.3 to 1% and

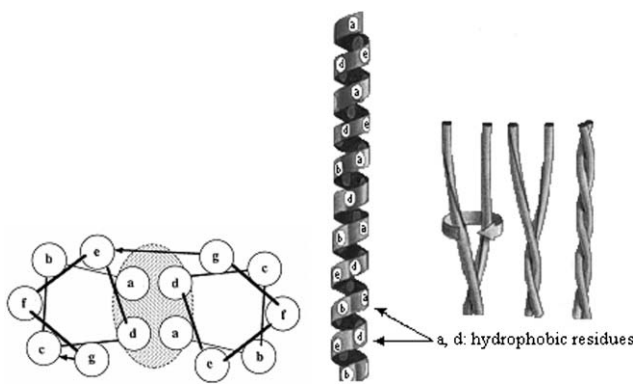


Fig. 13 The heptads (**abcdefg**) of amino acid residues of the α-helical segments of intermediate filaments, with **a** and **d** being the hydrophobic residues.[31]

the most frequent elements met are Ca, Cd, Zn, Cr, Cu, Fe, As, Si, Pb and Hg. Most of them are incorporated in hair from external sources and form coordination complexes with side groups of the proteins. Trace elements in hair have been used for the monitoring of environmental pollutants or as an indicator for certain diseases or poisoning.[43,44]

4. Conclusions

While discussing hair morphology and chemistry, one has to be aware that the hair is defined as being formed by the follicle and the shaft. There would be no organisation of the protein into a fibre without the formation of the material in the follicle and growing from the bulb through the skin pore (*cf.* Fig. 14). The birthplace of the fibre is, therefore, the follicle, and many investigations during the past years have addressed its biochemistry. The genome project also assists in enriching the knowledge about hair by pointing out the genes responsible for the cell differentiation and keratin formation.

Recent micro X-ray investigations show that the α-helices form an amorphous structure when produced in the bulb, but, along the first 1400 μm (which is the distance to the zone 5, Fig. 14), they organize to form the crystalline phase.[45] The process looks, at a first glance, like a lyotropic phase transition, a transition during which ordering effects are induced by changing the concentration; however, in the case of hair one

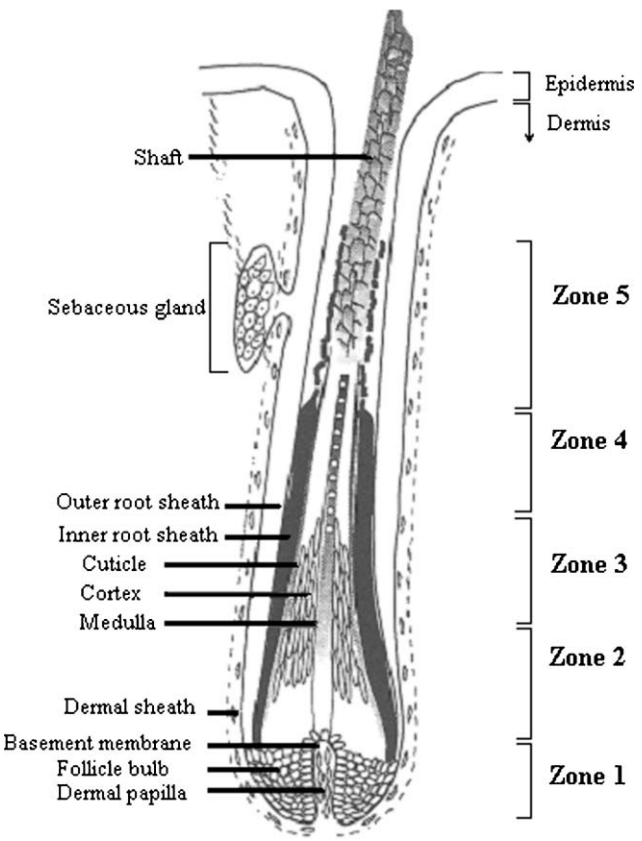


Fig. 14 The follicle and the shaft of a hair.[31] Zone 1—bulb zone (proliferation and differentiation); zone 2—elongation (fibril formation); zone 3—pre-keratinization (lateral aggregation); zone 4—hardening (keratinization); zone 5—post hardening (hard keratin).

has also to take into account that the fibre grows at a rate of about 300 μm per day (1 cm per month) and may assume that the flow of produced protein also plays a role in this organization.

The production of protein material in the bulb ranges from 6 months (the fine hairs of Cashmere goat) to 6–7 years (hair of most of mammalians, including humans). This is called the anagen (growing) stage of the hair and, at its end, the hair steps into the second stage, the regression (catagen) stage, for a short period of a few months. During this period the follicle stagnates, there is no more material produced and the contacts between the follicle walls and the fibre shaft cease. At the end of the catagen stage, the hair goes into the telogen (resting) stage, followed by the last one, exogen (shedding) phase and leaves the follicle once a light force (combing, brushing) is applied.[46] The follicle may re-enter the anagen stage after a while, resuming its protein production, or may remain dormant or even retract into the skin. The pathway of the follicle involves activity of hormones and the full mechanism is not fully deciphered, but the progress recorded so far allows to hope for achieving controlled growth of hair and eventually a solution to baldness.

The investigations of the amino acid sequences in hair offer results used not only for keratin fibre science, but also for the understanding of the behaviour of intermediate filaments and, by this, the behaviour of carcinoma cells, where these filaments play an important role.[47,48]

Summing up, the study and the understanding of hair morphology, chemistry and behaviour results in useful knowledge not only for textile and cosmetic science, but also about many of the most diverse processes of the living world.

References

1 J. H. Bradbury, The structure and chemistry of keratin fibres, *Adv. Protein Chem.*, 1973, **27**, 111.
2 D. B. Volkin and A. M. Klibanov, *J. Biol. Chem.*, 1987, **262**, 2945.
3 R. O. Herzog and W. Jancke, *Naturwissenschaften*, 1926, **14**, 1223.
4 W. T. Astbury and A. Street, *Philos. Trans. R. Soc. London, Ser. A*, 1931, **A230**, 75.
5 W. T. Astbury and H. J. Woods, *Philos. Trans. R. Soc. London, Ser. A*, 1933, **A232**, 333.
6 L. Pauling, R. B. Corey and H. R. Branson, *Proc. Natl. Acad. Sci. U. S. A.*, 1951, **37**, 205.
7 B. C. Powell and G. E. Rogers, The role of keratin proteins and their genes in growth, structure and properties of hair, in *Formation and structure of human hair*, ed. P. Jollès, H. Zahn and H. Höcker, Birkhäuser Verlag, Basel, Switzerland, 1997.
8 F.-J. Wortmann and K.-H. Phan, *Wool Record*, 2004, **10**, 51.
9 J. A. Maclaren and B. Milligan, *Wool science. The chemical reactivity of the wool fibre*, Science Press, Marrickville, NSW, Australia, 1981, pp. 2–4.
10 M. G. Dobb, *J. Text. Inst.*, 1970, **61**, T232.
11 F.-J. Wortmann and H. Deutz, *J. Appl. Polym. Sci.*, 1998, **68**, 1991.
12 J. W. S. Hearle, *Wool Technol. Sheep Breed.*, 2003, **51**, 95.
13 T. Audesirk and G. Audesirk, *Biology*, Prentice Hall, Upper Saddle River, NJ, USA, 1999.
14 D. A. D. Parry and P. M. Steinert, Intermediate filaments: molecular architecture, assembly, dynamics and polymorphism, *Q. Rev. Biophys.*, 1999, **32**, 99.
15 H. Zahn, J. Föhles, M. Nienhaus, A. Schwan and M. Spei, *Ind. Eng. Chem. Prod. Res. Dev.*, 1980, **19**, 496.
16 M. Feughelman, *J. Appl. Polym. Sci.*, 2002, **83**, 489.
17 M. G. Dobb, F. R. Johnston, J. A. Nott, L. Oster, J. Sikorski and W. S. Simpson, *J. Text. Inst. Trans.*, 1961, **52**, T153.
18 L. N. Jones, N. R. Watts, F. P. Booy, A. C. Steven and D. A. D. Parry, *Asian–Australas. J. Anim. Sci.*, 2000, **13**, 120.
19 B. Ahmadi and P. T. Speakman, *FEBS Lett.*, 1978, **94**, 365.
20 L. N. Jones and F. M. Pope, *J. Cell Biol.*, 1985, **101**, 1569.
21 C. R. Robbins, *Chemical and physical behaviour of human hair*, Springer Verlag, New York, 1994, p. 35.
22 L. Zecca, D. Tampellini, M. Gerlach, P. Riederer, R. G. Fariello and D. Sulzer, *Mol. Pathol.*, 2001, **54**, 414.
23 J. McGinness, P. Corry and P. Proctor, *Science*, 1974, **183**, 853.
24 R. A. Nicholaus, in *Advances in biology of skin, the pigmentary system, vol. 8*, ed. W. Montagna and F. Hu, Pergamon Press, New York, 1966, pp. 293–312.
25 K. C. Littrell, J. M. Gallas, G. W. Zajac and P. Thiyagarajan, *Photochem. Photobiol.*, 2003, **77**, 115.
26 L. J. Wolfram and K. J. Hall, *J. Soc. Cosmet. Chem.*, 1975, **26**, 247.
27 A. Berek, H. Zahn and S. Schwartz, *Text. Prax. Int.*, 1982, **37**, 621.
28 J. B. Speakman and C. A. Cooper, *J. Text. Inst.*, 1936, **27**, T191.
29 W. J. Onions, *Wool, an introduction to its properties, varieties, uses and production*, Ernest Benn Ltd., London, 1962.
30 T. Vickerstaff, *The physical chemistry of dyeing*, Oliver and Boyd, London, 1954, p. 349.
31 H. Zahn, K. Schaeffer and C. Popescu, Wool from Animal Sources, in *Biopolymers*, ed. Alexander Steinbüchel and Stephen R. Fahnestock, Wiley-VCH Verlag GmbH & Co., Germany, 2003, Vol. 8: Polyamides and complex proteinaceous materials II, ch. 7, pp. 155–202.
32 M. F. Perutz, *Faraday Discuss.*, 1992, **93**, 1.
33 N. Nishikawa, Y. Tanizawa, S. Tanaka, Y. Horiguchi and T. Asakura, *Polymer*, 1998, **39**, 3835.
34 Charles Nessler, *GB Patent* No. 20597, 1909.
35 D. W. Cannell, *Clin. Dermatol.*, 1988, **6**, 71.
36 P. L. Le Roux and J. B. Speakman, *Nature*, 1955, **176**, 510.
37 O. Ripa and J. B. Speakman, *Text. Res. J.*, 1951, **21**, 215.
38 D. G. Phillips, J. J. Warner, *Patent* No. WO91/02835, 1991.
39 L. J. Wolfram and M. K. O. Lindemann, *J. Soc. Cosmet. Chem.*, 1971, **22**, 839.
40 A. P. Negri, H. J. Cornell and D. E. Rivett, *Text. Res. J.*, 1993, **63**, 109.
41 F. H. C. Crick, *Nature*, 1952, **170**, 882.
42 F. H. C. Crick and J. C. Kendrew, *Adv. Protein Chem.*, 1957, **12**, 133.
43 I. Obrusnik, *J. Hyg., Epidemiol., Microbiol., Immunol.*, 1986, **30**, 11.
44 A. Sukumar and R. Subramanian, *Ind. Health*, 2003, **41**, 63.
45 M. Er Rafik, F. Briki, M. Burghammer and J. Doucet, *J. Struct. Biol.*, 2006, **154**, 79.
46 K. S. Stenn and R. Paus, *Physiol. Rev.*, 2001, **81**, 449.
47 F. C. S. Ramaekers, D. Haag, A. Kant, O. Moesker, P. H. K. Jap and G. P. Vooijs, *Proc. Natl. Acad. Sci. U. S. A.*, 1983, **80**, 2618.
48 G. S. Sidhu, P. Chandra and N. D. Cassai, *Ultrastruct. Pathol.*, 2005, **29**, 287.
49 Source: The Online Biology Book, http://www.estrellamountain.edu/faculty/farabee/biobk/biobooktoc.html.