Mimicking biopolymers on a molecular scale: nano(bio)technology based on engineered proteins

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Proteins are ubiquitous biopolymers that adopt distinct three-dimensional structures and fulfil a multitude of elementary functions in organisms. Recent systematic studies in molecular biology and biotechnology have improved the understanding of basic functional and architectural principles of proteins, making them attractive candidates as concept generators for technological development in material science, particularly in biomedicine and nano(bio)technology. This paper highlights the potential of molecular biomimetics in mimicking high-performance proteins and provides concepts for applications in four case studies, i.e. spider silk, antifreeze proteins, blue mussel adhesive proteins and viral ion channels.

Keywords: antifreeze proteins; biosensors; molecular biomimetics; Mytilus edulis foot proteins; spider silk proteins; viral ion channels

1. Introduction

The advances in molecular biology and nanoscale engineering broaden the spectrum of biomimetics to the molecular scale (Ball 2001), including biopolymers such as polypeptides (proteins), which are nanoscaled building blocks abundant in all organisms. Since the evolution of life is intimately connected to proteins, they inherit diverse elementary functions ranging from DNA replication to catalysis, transport and communication, as well as morphological stability (Whitford 2005). Proteins are synthesized from combinations of up to 19 amino acid monomers and one imino acid monomer in template-directed reactions catalysed by small cellular units called ribosomes within cells. These monomers, or residues, are linked via amide (peptide) bonds,

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yielding monodisperse linear polymers with a specific sequence of monomers (i.e. the primary structure) (Kozak 1983). The specificity and diversity of a protein’s function are based on the unique sequences in the amino acid chains that assemble or fold to compact domains. The folded domains can provide binding and/or catalytic sites for a variety of physiological functions, or serve as modules for building blocks of larger assemblies such as virus particles or muscle fibres (Branden & Tooze 1991). Protein folding itself is a self-assembly process based on primary structure, favourable thermodynamics and appropriate kinetics (Anfinsen 1973). The main driving force behind protein folding in aqueous solutions is the reduction of free energy by excluding the hydrophobic residues from water (Vauthey et al. 2002). Protein structures are classified into four categories (Whitford 2005). While the primary structure of the protein is the linear sequence of amino acids along the polypeptide chain, the secondary structure refers to distinct local conformations. In globular proteins, there are predominantly three types of thermodynamically stable secondary structures: α-helices; β-sheets; and turns. The tertiary structure represents the three-dimensionally folded polypeptide chain. These chains are stabilized by non-covalent bonds such as electrostatic and van der Waals interactions, hydrophobic interactions, hydrogen bonds and covalent bonds such as disulphide bridges. Finally, the structure formed upon the interaction between the individual tertiary structures of more than one polypeptide chain is known as a quaternary structure.

Recent systematic studies in molecular biology and biotechnology have improved the understanding of basic architectural and assembly principles of proteins. This makes them attractive candidates as concept generators for development in material science, biomedicine and nano(bio)technology. This paper focuses on the potential of molecular biomimetics in the engineering of high-performance proteins highlighted by four case studies, i.e. spider silk as an outstanding fibre material, antifreeze surface coatings, blue mussel adhesive proteins and viral ion channels for biosensors and molecular switches.

2. Molecular biomimetics of proteins: four case studies

(a) A natural high-performance protein fibre: spider silk

(i) Natural spider silks

Silks are protein-based fibres made by arthropods for a variety of task-specific applications, e.g. protective cocoons, catching and wrapping prey and even as a means of transport (Lin et al. 1995; Heim et al. 2009). They are typically composite materials formed of silk proteins and associated molecules, such as glycoproteins, lipids and salts (Winkler & Kaplan 2000; Sponner et al. 2005). Insects, such as silkworms, use their silk to produce a protective cocoon during the metamorphosis of the caterpillar into a moth, which is composed of proteinaceous silk components called fibroin (Inoue et al. 2000). Humans have harvested silkworm silk for millennia to produce textiles, since silkworms can easily be farmed.

Spiders are able to produce a variety of silk types that have different mechanical properties. Approximately half of the 40 000 spider species known build webs, of which more than 130 different architectures have been described.
As an example, the orb web of the European garden spider *Araneus diadematus* consists of four different types of silk (Römer & Scheibel 2008). The frame and radii of orb webs as well as the drag line are made of strong and rather rigid silk whose proteins are produced in the major ampullate glands (MA silk). The capture spiral of an orb web comprises fibres of only one type of protein that is produced in the flagelliform gland (Flag silk). These fibres are highly extensible (up to 300%) and perfectly dissipate the impact energy of prey caught in mid-flight (Vollrath 2000). The web also incorporates minor ampullate (MI) silk for an auxiliary spiral, and pyriform silk as an attachment cement. Furthermore, *A. diadematus* produces aggregate silk as a sticky hygroscopic coating of the capture threads. Other silks include tough eggshell silk, a soft lining silk inside the eggshell and for wrapping prey (Römer & Scheibel 2008). Spider silk is an outstanding fibrous proteinaceous biopolymer, combining high tensile strength and elasticity, leading to a toughness that is two to three times that of synthetic fibres available (Gosline et al. 1999). Natural spider silk is also antimicrobial, hypoallergenic and completely biodegradable (Kaplan et al. 1993), causing interest to industrial researchers seeking for new materials and new applications.

(ii) Assembly of spider silk proteins

Spider silk primarily consists of proteins that possess large amounts of non-polar and hydrophobic amino acids such as glycine (Gly) or alanine (Ala) (Vollrath 2000; Heim et al. 2009). Their large core domains, in particular, contain highly repetitive amino acid sequences composed of short polypeptide stretches of approximately 10–50 amino acids, which often account for more than 90 per cent of the whole spider silk protein. Alanine-rich blocks are known to form β-sheet structures in assembled fibres being responsible for the high tensile strength of MA silk, whereas glycine- and proline-rich blocks in flagelliform and MA silk presumably form 3_10-helices and β-turn spirals imparting elasticity, respectively (Hayashi & Lewis 1998; Rising et al. 2005; Hardy et al. 2008). The non-repetitive terminal domains of the proteins comprise approximately 100–200 amino acids and are hypothesized to play a role in the assembly of spider silk proteins into fibres during spinning (Huemmerich et al. 2004a; Exler et al. 2007). Different types of silk have different structural distributions, e.g. different compositions of crystalline and hydrogel-like parts. Whereas rigid and strong silk proteins such as MA silk contain a large amount of crystalline structures, the much more flexible Flag silk consists almost exclusively of amorphous hydrogel-like regions (Kaplan et al. 1993).

The process of spider silk assembly starts with the synthesis of spidroin proteins in specialized cells and their secretion in the lumen of spinning glands, where a highly concentrated spinning dope solution is stored (figure 1). Upon initiation of thread formation, the dope is directed from the gland through a narrow valve into the spinning duct resembling an ion exchange channel in which a liquid–liquid phase separation followed by a liquid–solid phase transition takes place (Scheibel 2004; Exler et al. 2007; Heim et al. 2009). In the duct, controlled changes of several environmental conditions induce protein aggregation: the pH drops from 7.2 to 6.4, and sodium and chloride ions are exchanged for more kosmotropic potassium and phosphate ions, combined with water extraction, leading to the so-called salting-out effects accompanied by the folding and
formation of the silk thread (Vollrath & Knight 2001; Rammensee et al. 2008). The primary structure of spider silk proteins produced in the various glands is typically amphiphilic, which is thought to be crucial for phase separation during the spinning process. Mechanical stress such as shear-induced elongation of the spinning dope during passage through the duct encourages bonding interactions of the protein building blocks (Heim et al. 2009). Mechanical drawing of the silk thread by the hind legs or gravity results in rapid assembly of the silk fibre. Two theories exist concerning the molecular process inside the spinning duct. The first theory was deduced from in vivo studies of spider silk and supports a liquid-crystalline behaviour of the underlying silk proteins (Vollrath & Knight 2001), whereas the second theory is based on an in vitro analysis of the silkworm silk assembly and favours a micellar organization of the proteins, before elongation induced by laminar forces finally leads to thread assembly (Jin & Kaplan 2003). The aforementioned amphiphilic pattern of silk protein is reminiscent of biological membranes and might be responsible for the formation of micelles postulated as intermediate structures during thread assembly.

(iii) Recombinant silk proteins, technical fibre spinning and applications

Since it is impossible to farm most spiders in a large scale due to their cannibalistic nature, a number of biotechnological methods for recombinant production of spider silk proteins have been applied to mimic natural spider silk in technical applications (Vendrely & Scheibel 2007). Several aspects hamper an easy and straightforward approach to achieve recombinant spider silk proteins: the size of the underlying silk genes (up to 15 Mbp) and the highly repetitive sequences, whose analysis often yields incorrect or unreliable results; still limited knowledge of the cDNA/genes that encode spider silk proteins; and different codon usage between spiders and typical industrial production hosts for gene transformation such as the bacterium Escherichia coli.
Some of these problems have been solved by expressing authentic spider cDNA in eukaryotic expression systems such as yeast, or transgenic plants such as potato or tobacco (Scheller et al. 2001). Other expression systems for the direct transformation of spider MA silk cDNA fragments include bovine mammary epithelial cells, hamster kidney cells, baculovirus-infected insect cells and mammary glands of transgenic goats, albeit with a low protein yield (Lazaris et al. 2002; Huemmerich et al. 2004b; Miao et al. 2006). To achieve stable silk protein production with efficient yields, synthetic silk genes have been designed using a cloning strategy that is based on a combination of synthetic DNA modules and PCR-amplified authentic gene sequences. The protein sequences of single, repetitive silk motifs were reversely transcribed into oligonucleotides using E. coli’s codon usage (Huemmerich et al. 2004a; Vendrely et al. 2008). Thereby, the sequences of these engineered proteins are not identical but mimic those of the established, natural silk fragments. The modular system enabled the production of specifically engineered proteins with tailor-made properties for experimental analysis (Rammensee et al. 2008; Römer & Scheibel 2008).

Several attempts have been made to spin recombinant spider silk proteins, including hand drawing (Shao et al. 2003; Teule et al. 2007), wet spinning and electrospinning (Seidel et al. 1998; Lazaris et al. 2002; Wang et al. 2004). In most of these cases, the mechanical properties of such silk fibres were not comparable with the natural template. This mismatch could be due to the poor quality and composition of the artificial silk protein material as well as an inadequate spinning technique. Several aspects play a critical role in silk assembly: the protein composition of the spinning dope; the phase separation process in the spinning duct; and several mechanical parameters (Heim et al. 2009). In a recent study, research in the Fiberlab tried to mimic the natural spinning process in a biomimetic approach with microfluidic devices (Rammensee et al. 2008). In a series of experiments, different parameters such as pH, ion abundance and concentration and drawing speed were tested using recombinantly produced silk proteins. This technique is the basis for a novel biomimetic spinning process, leading to engineered silk fibres comparable to that of natural silk, with mechanical properties close to the natural model (Heim et al. 2009).

Protein engineering allows the generation of new proteins with additional features not found in natural silk proteins. The incorporation of specific amino acids influences protein solubility, allows triggering of silk assembly or generates new functional properties due to chemically active side chains (Winkler et al. 2000; Scheibel 2005). Moreover, it is possible to assemble spider silk proteins not only as a linear thread, but also as films, hydrogels, capsules, nanofibrils and nanovesicles, enabling various biomedical, cosmetic and technical applications (Scheibel 2005; Vendrely & Scheibel 2007; Römer & Scheibel 2008).

As silk combines excellent mechanical properties, biocompatibility, antimicrobial properties and slow biodegradability, engineered silk proteins have a huge potential as materials for tissue engineering and guided tissue repair in biomedical applications. Spider silk fibres could be applied in textiles that demand high toughness in combination with stretchiness, e.g. in parachutes. The ability of silk proteins to self-assemble potentially allows their employment in micromechanical and nanoelectronic applications, e.g. nanowires or electroconductive surface coatings.
(b) Survival in extreme environments: antifreeze proteins

(i) Anti-icing mechanism in engineering and nature

The control of ice formation on interfaces is crucial in both natural and technical systems. Ice formation on surfaces of technical transport vehicles, e.g. aircraft, rail, ships and cars, or electrical and cooling systems can cause irreparable damage and severe accidents (Fitt & Pope 2001). Icy surfaces may function as an unwanted electric insulator causing higher energy consumption. The removal of ice and maintenance and repairs of plants are both cost intensive and time consuming, and cause undesirable downtime and profit deficits. Two methods are generally distinguished to prevent icing and ice crystal formation in technical systems, i.e. anti-icing (prevention of ice formation) and de-icing (removal of ice structures) techniques. These can be further divided into passive methods, e.g. recycling waste heat from engines or infrared (IR) radiation absorbing coatings (Lacroix & Manwell 2000), and active methods, e.g. heat generation to melt the ice. Other anti-icing methods use surface deformation and chemical substances as freezing point depressants such as salts, glycerols, sugars and alcohols (Kiernan 1995). The methods mentioned may have several drawbacks including damage of surfaces, corrosion, consumption of extra energy and usage of environmentally unfriendly chemicals.

In nature, organisms adapt and evolve in order to survive and proliferate in their respective environment. Organisms without active thermoregulation, such as fishes, reptiles, amphibians and insects, adapt their body temperature to the environment, i.e. they are ectothermic. These organisms have managed to survive in temperature regimes below 0°C via two basic strategies: freeze avoidance and freeze tolerance (Margesin et al. 2007). Freeze avoidance involves low molecular mass substances, such as sugars and polyols (e.g. glycerol), that lower the freezing temperature of the body fluids through colligative effects. Additionally, certain proteins named antifreeze proteins (AFPs) that inhibit ice crystallization are often produced (DeVries 1971). Freeze-tolerant animals endure ice formation in extracellular fluid spaces but defend the liquid state of cytoplasm, e.g. by ice-nucleating proteins (Duman 2001). Since the first studies on arctic fishes in the 1950s, it has been known that certain animals, microbes and plants can survive fully functional at temperatures as low as −30°C with the help of AFPs (Scholander et al. 1957; Griffith et al. 2005; Margesin et al. 2007).

(ii) Antifreeze proteins

The physical process of freezing point depression generally depends on the amount of molecules dissolved in a liquid (colligative property; Atkins & de Paula 2002). AFPs show a different behaviour: the body fluid of organisms normally starts to freeze at −0.7°C; however, AFPs are able to effectively suppress ice formation by depressing the freezing point of the fluid, while the melting point remains approximately 0°C (DeVries 1971; Hew & Fletcher 1985). This behaviour is called a constitutive property and depends only on the structure of the molecules. The gap between the melting point and the freezing point is called thermal hysteresis (Barrett 2001; figure 2). AFPs are able to depress the freezing point 200–500 times more effectively by their constitutive property than via their possible colligative effects. Some of them are able to...
depress the freezing point as low as \(-11^\circ C\) \textit{in vitro}, depending on the type and structure of the AFP (Duman et al. 2004). All AFPs described so far are surface-active materials and influence the ice crystal size, shape, growth and aggregation by binding to very small ice crystals called ice nuclei. The nature of binding depends on the distinctive structure and amino acid sequence of the protein and seems to include both hydrophobic and hydrophilic sides (Knight 2000; Graether et al. 2001). The inhibitory effect of AFPs on ice formation is an entirely kinetic (non-equilibrium) phenomenon (Westh et al. 1997); however, different hypotheses exist on the detailed mechanism of how the AFPs lower the freezing point: when the AFPs interact with ice surfaces, new crystal growth can only occur between the binding sites of the proteins, leading to a curvature/bend of this area. Since the vapour pressure and the chemical potential are related to the surface curvature (Gibbs–Thomson effect), bending causes a higher vapour pressure due to a larger surface in comparison with the volume, and results in the observed freezing point depression (Knight 2000). A recently published molecular dynamics (MD) study, however, showed that the AFPs from animals are able to interact with the ice in a stable way only if the hydrophobic residues of the proteins are bound to the hydrophilic ice (Nada & Furukawa 2008).

The protein sequence and structures of AFPs from arctic fishes and some insects are well characterized, and several other proteins with antifreeze properties are known (figure 2). Fish AFPs are divided into five major groups: AFP type I–IV and the antifreeze glycoproteins (AFGP; Kristiansen & Zachariassen 2005). Type I–IV AFPs display special protein folding and conformational behaviour, which allows direct interaction with the surface of ice crystals. Type I AFPs have been intensively studied because they can be isolated, recombinantly expressed (e.g. in yeast or bacteria) or chemically synthesized (Harding et al. 1999). They are small monomeric proteins with molecular weights (MWs) of approximately 3–5 kDa and contain a high amount of the non-polar amino acid alanine, which, when in repetitive motifs of 11 or

![Figure 2](http://rsta.royalsocietypublishing.org/)

**Figure 2.** (a) Effect of the thermal hysteresis of AFPs with constitutive properties compared with colligative properties. (b) Three-dimensional models of AFPs based on the structural data of corresponding proteins from the RCSB protein database. Modelling was performed with the software YASARA.
more amino acids, is known to form $\alpha$-helical structures in solution (Sicheri & Yang 1995; Graether et al. 2001; Marshall et al. 2004). Type II AFPs have a MW range of 11–22 kDa and display a more complex globular structure involving $\alpha$-helices, $\beta$-sheets and $\beta$-strands with five intramolecular cysteine–cysteine disulphide bridges (Ng & Hew 1992; Gronwald et al. 1998). The type III AFPs have a globular structure that differs completely from other AFP structures, i.e. a non-helical structure (Sonnichsen et al. 1993). The type IV AFPs contain anti-parallel ordered $\alpha$-helices with MW of approximately 12 kDa and a high amount of the polar amino acid glutamine (Deng et al. 1997). The AFGP contain oligosaccharide molecules covalently attached to the side chains of certain amino acid residues. They display the broadest spectrum of MWs within fish AFPs (Knight et al. 1984). Additional AFP isoforms from insects with interesting properties have been discovered (Duman 2001). Insect AFPs display a higher thermal hysteresis compared with fish AFPs and can be up to 20 times more effective, as shown for the spruce budworm Choristoneura fumiferana (Tyshenko et al. 1997). Structural analysis of this protein suggested an unusual structure of a left-handed $\beta$-helix with 15 amino acid residues per turn (Li et al. 2005), while that from the beetle Tenebrio molitor displays also a regular $\beta$-helix with 12 amino acid loops and tandem 12-residue repeats (Liou et al. 2000).

(iii) Protein-based anti-ice coating: concepts and applications

The described features of AFPs are very interesting for nano(bio)technological applications, and the feasibility of both anti-ice and ice nucleation coatings has been demonstrated in proof-of-concepts studies (Garner & Harding 2007). Wierzbicki et al. (2000) described the structure–function relationship of a de novo synthesized 43 residue alanine–lysine-rich antifreeze polypeptide that is able to bind to designated ice planes along a specific direction. Zwieg et al. (2007) described a novel protein-based ice-nucleating coating containing locally isolated nucleation points in a low surface energy matrix that was synthesized by a sol–gel process. In addition to ice nucleation, this coating also displayed improved ice-repellent properties compared with commercial coatings. Another biomimetic approach to anti-icing surfaces has been performed with superhydrophobic coatings inspired by the sacred lotus leaf (Solga et al. 2007). The biomimetic surfaces based on commercially available lotus-effect paints were good at self-cleaning, but showed poor anti-icing properties (V. Stenzel 2007, unpublished results). It has also been shown that AFGP act as cryoprotectants for cell membranes (Inglis et al. 2006). However, a lack of detailed knowledge about how AFPs function in a given lipid membrane system hampers progress towards the development of molecules for cold storage of cells, tissues and organs.

A feasibility study to modify lacquer surfaces with AFPs was conducted at the Fraunhofer IFAM, Germany (Grunwald & Rischka 2008). Winter flounder and the European fir budworm were chosen as suitable AFP model organisms. Defined peptide sequences were produced by solid-phase peptide synthesis on a laboratory scale. Bonding of the artificial AFP residues to several commercially available lacquer systems was achieved using three different strategies. The first approach involved spraying of an aqueous protein solution onto the lacquer using an ultrasound nebulizer. The coating system consisted of an epoxy resin and a polyamine hardener. The amino groups of the proteins reacted with the epoxide
groups on the lacquer surface and became incorporated into the polymer. In the second approach, the AFPs were attached by photochemical means, using a photochemically active molecule integrated into the lacquer system. In the third approach, the peptides were attached via linker molecules to the lacquer, thereby displaying the AFP residues on the surface. This approach proved to be very promising. Reference tests were carried out with the different coating set-ups in a frosting chamber at controlled air flow temperatures. Considerably less ice formed on the AFP-functionalized surfaces compared with the control, while a non-uniform coating also resulted in non-uniform frosting patterns. The results demonstrate both the feasibility and the enormous potential of biomimetic antifreeze coatings based on AFPs.

(c) Adhesion under water: adhesive proteins of blue mussel byssus plaque

(i) Adhesion in underwater applications

Gluing is one of the most important methods of bonding technology; however, the sensitivity of glues to water is a big challenge for industry concerning underwater applications. Whereas traces of water in the form of air moisture or layers of water molecules absorbed as fluid films on the bonding surfaces are mandatory for the polymerization and physicochemical interactions of certain technical adhesives, such as cyanoacrylates, larger amounts of water may restrict their functionality (Waite 1987). The cohesion (internal bonding forces) of an adhesive is weakened by swelling, plastification, erosion and hydrolysis upon interaction with water, and adhesion (bonding forces between adhesive and surface) is dramatically reduced as the polar groups abundant in the adhesive interact with the polar water molecules.

Naturally occurring bioadhesives have evolved to cope with the phenomena described above. One pertinent example of a marine organism with highly efficient attachment abilities is the blue marine mussel *Mytilus edulis*. It attaches to a variety of surfaces via a byssus, of which an adhesive cement (plaque) is glued to the substrate (figure 3). The threads are dominated by collagen–silk or collagen–elastin structural proteins, and the cement is composed of several polyphenolic adhesive proteins called *M. edulis* foot proteins (Mefp; Sagert et al. 2006). Upon initiation of the gluing process, a bowl-shaped distal depression at the foot tip is pushed against the substrate by muscle action until the ceiling of the depression touches the surface. Then, the depression is lifted and adhesive precursor molecules are applied from a large phenol gland via conducting tubules and pores into the cavitation (Waite 1987).

(ii) Chemical composition of the natural mussel adhesive

Six different types of adhesive Mefp have been identified so far; among these, the polyphenolic Mefp-1 was the first discovered byssus protein. The Mefp-1 consists of several repeats of a decapeptide sequence (Waite 1983). Each decapeptide subunit contains post-translationally modified amino acid residues, e.g. hydroxyproline (Hyp) and dihydroxyphenylalanine (DOPA). The Mefp-1 has a DOPA content of up to 15 mol% and interacts strongly with polar moieties abundant on surfaces due to the large amount of polar amino acid residues in the sequence. However, a recent study showed that Mefp-1 is not responsible for the...
adhesive properties of the byssus plaque, but acts as a protective coating for both
the byssus plaque and thread (Lin et al. 2007). Mefp-2 has a significantly lower
DOPA content than Mefp-1 and has no other post-translationally modified
amino acid residues; it contains a high amount of the amino acid cysteine (up to
6–7 mol%) and is responsible for the foamy morphology and structural
stabilization of the byssus cement (Rzepecki et al. 1992). Mefp-3 is the smallest
protein of the Mefp family and has the highest DOPA content of 15–30 mol%
(Papov et al. 1995; Waite & Qin 2001). Similarly, Mefp-5 has a high DOPA
content and also contains a significant amount of post-translationally
phosphorylated serine residues (Waite & Qin 2001). Both proteins act as
adhesion promoters and support the bonding of the plaque to the substrate
(Holten-Andersen & Waite 2008). Mefp-4 acts as a linker protein in the junction
region between the collagen fibres of the distal byssus thread and the plaque
proteins (Zhao & Waite 2006a). A sixth type of mussel foot protein (Mcfp-6) was
isolated from the species Mytilus californicus, which may mediate the coupling of
abundant surface proteins with the plaque proteins by cysteiny1-DOPA
crosslinks (Zhao & Waite 2006b). The high DOPA content in most of the
mussel proteins is remarkable. DOPA is a key amino acid for adhesion and
cohesion due to its manifold possible chemical reactions (Yu et al. 1999). The
catechol moiety of DOPA has the ability to form extremely stable metal
complexes, while hydroxyl groups are hydrogen bond donors and acceptors. The
covalent crosslinkings crucial for the cohesion of the mussel adhesive proteins are
established by radical dimerization of two catechol moieties to form di-DOPA
and by 1,4-Michael additions of amino groups of the lysine, histidine and cysteine
residues (Sagert et al. 2006). Recent publications have shown that metal ions,
e.g. manganese or iron, may also support crosslinking within the Mefp adhesive
(Sever et al. 2004).

Figure 3. Blue mussel Mytilus edulis adhesion system: a scanning electron micrograph of the
binding site of a byssus thread, and a scheme of the byssus foot, thread and plaque.

Phil. Trans. R. Soc. A (2009)
(iii) Mussel protein-based synthetic adhesives: concepts and applications

Purified mussel adhesive proteins have been investigated on pig skin tissue and bonding strength was tested (Ninan et al. 2003). Despite some limitations for possible clinical use, the results of this study suggested the potential use of Mefp as an adhesive for soft tissues. Artificial peptide-based adhesives derived from Mefp-1 were generated by polycondensation of 10 decapeptide units and tested in comparison with polylysines on different metal surfaces in dry state (Yamamoto 1987). However, the polyllysines outperformed the synthetic polydecapeptide with the highest tensile strength on iron substrates. In other studies, artificial co-polypeptides (Yu & Deming 1998) and ter-polypeptides (Tatehata et al. 2000) containing DOPA and lysine residues were synthesized and subsequently cross-linked by addition of several oxidation agents. A significant improvement in the bonding strength of these systems was not observed, since Mefp-1 was shown to form rigid coatings, but no bridging to the substrate surface, which would improve the adhesion properties (Lin et al. 2007; Holten-Andersen & Waite 2008).

Up to now, no synthetic adhesive is available comparable to the complex natural tenacious holdfast system of the mussel plaque (Silverman & Roberto 2007). Recently, Lee et al. (2007) showed that a combination of nanostructured polymers with acrylates containing catechol groups from mussel proteins improved the adhesion under water of highly fractal surfaces based on the reversible adhesion principle of gecko setae. Mefp-1-based coatings with antifouling properties have also been successfully generated by conjugation of Mefp-1 decapeptides to polyethylene glycol (PEG) on titanium or gold surfaces (Dalsin et al. 2003).

Hybrid systems containing Mefp-1 decapeptides and synthetic polymers have been used for the production of hydrogels for medical applications. The DOPA residues were conjugated to PEG, resulting in improved adhesive and cohesive properties (Lee et al. 2002). Other hybrid systems containing Mefp-based peptides and synthetic polymers, e.g. different acrylates and a mixture of these, were synthesized at Fraunhofer IFAM by solid-phase peptide synthesis. These adhesives were tested on different substrates and displayed improved tensile shear strength compared with pure acrylate systems. The results encourage a potential use of Mefp-based hybrid adhesives in medical applications, where epoxy or cyanoacrylate resin-based adhesives such as hydroxyethyl-methacrylate (HEMA) are the state-of-the-art adhesives despite their limited biocompatibility (Geurtsen 2000). Chemically engineered Mefp-based proteins can be linked to a variety of suitable polymer backbones to form hybrid polymers, followed by functionalization to promote the accretion of human cells, that may lead to applications combining improved adhesion and tissue regeneration. To this end, Fraunhofer IFAM is currently developing an innovative Mefp-based adhesive for dental implants.

(d) Signalling at the nanoscale: proteins as molecular switches and biosensors

(i) Proteins as natural nanobiosensors

A fourth case study focuses on ion channels that currently receive a lot of attention (Bayley & Cremers 2001; Banghart et al. 2006), since they are important proteins in membranes of all types of living organisms and catalyse the
selective and highly regulated transport of ions across membranes (Hille 2001). Ion channels are *per se* nanoscale biosensors, since they consist of building blocks of an electrical lead element operator side and several specific sensory domains of high sensitivity. This modular building principle enables them to selectively register chemical and physical parameters and transform them into electrical signals (Thiel & Moroni 2004). The fact that these proteins can be gated, i.e. can switch between an active ion conducting and an inactive non-conducting state in response to chemical ligands and physical signals, has fostered the idea that channel proteins can be used as molecular switches and biosensors in artificial systems. Several examples have already successfully shown proof of principle that the structural properties of ion channels can be altered by protein engineering in such a way that they acquire new functional properties. One notable example is the successful construction of a functional light-gated ion channel (Volgraf et al. 2006).

(ii) **Viral ion channels**

A core element of a designed channel is the pore module, which should be gateable, and this gating mechanism should be easily linked to a regulatory module. The building principles of this basic pore element can be derived from different approaches. Guided by the sequence information of ion channels from bacteria and eukaryotes, Lear et al. (1988) constructed very simple α-helices using only the amino acid residues serine (Ser) and leucine (Leu) in alternating sequence. These artificial proteins had channel-like activity when the helices were long enough to span the whole membrane bilayer, although without ion selectivity or gating of a channel.

An alternative approach tries to mimic the structure–function relationship of viral proteins. Viruses are true followers of the ‘Bauhaus’ concept of architecture and are masters in minimal design. The structural protein M2 of virus influenza A was the first viral-coded ion channel discovered, and has been intensively examined due to its potential as a target for antiviral drugs (Pinto et al. 1992; Pinto & Lamb 2004). M2 protein is a tetramer in its active form and the space between the monomers most probably functions as a channel pore (Stouffer et al. 2008; Schnell & Chou 2008). A single histidine residue (His37) in the pore-lining part of the channel is crucial for a H⁺ selectivity and ion transport. Another amino acid residue (tryptophan, Trp41) in the transmembrane domain in the vicinity of His37 is responsible for the pH-dependent gating mechanism of the M2 channel. The two amino acid residues are able to sterically interact due to rotation by a single turn of the α-helix induced by protonation of His37 at pH values lower than 7 on one side of the channel, opening the pathway for ion transport. Since the seminal discovery that M2 resembles an ion channel, a number of viral proteins with experimentally confirmed channel function such as M2 have been described (table 1). The design principles of the M2 channel protein are beautiful by virtue of their simplicity, since they can be reduced to some basic physicochemical principles. It is the nature of virus particles that their structural proteins must be very stable, and synthesis of viral proteins has to be rather easy in many types of cells. These features are highly interesting for nano(bio)technological applications and make viral channels an excellent source of information on essential architectural principles of functional channels (Fischer & Sansom 2002).
The viral proteins with channel function known so far have only very few building principles in common. Bioinformatic analysis of the virus channel protein sequences revealed that the great majority shows no similarity to any of the prokaryotic or eukaryotic ion channels (Fischer & Sansom 2002). In sharp contrast, the two channel proteins Kcv and Kesv, coded by algae viruses, share significant homology with the pore elements of known K_+ channels. The viruses of the unicellular green alga genus Chlorella contain double-stranded DNA (Van Etten 2003), and genomic sequencing of these viruses exhibited an open reading frame with all the hallmarks of a K_+ channel present in all forms of life, i.e. the selectivity filter sequence, two aromatic amino acid residues upstream of the filter, and two transmembrane domains (Plugge et al. 2000; figure 4). The most striking feature of the fully functional viral K_+ channel Kcv is its size of only 94 amino acids (Gazzarrini et al. 2004; Hertel et al. 2006; Shim et al. 2007). Besides an appreciable selectivity among cations, the Kcv channel remarkably reveals some voltage sensitivity, although a significant voltage sensor is absent (Plugge et al. 2000).

(iii) Biosensors and molecular switches: concepts and applications

The viral Kcv channel protein is not only functional within the natural membrane, but can be functionally reconstituted in planar lipid bilayers. This has been achieved in two different approaches: heterologous expression in the yeast Pichia pastoris (Pagliuca et al. 2007) or via in vitro translation (Shim & Gu 2007), followed by purification and reconstitution in lipid bilayer sandwich systems that allowed continuous single Kcv channel recordings lasting for several days as a robust and portable device. The purified proteins revealed the
exceptional stability of the channel complex and produced typical ion channel fluctuations in lipid bilayer experiments. Single channel data showed a very high unitary conductance of approximately 170 pS in 100 mM K\(^+\), which guarantees a high signal-to-noise ratio. These approaches demonstrate that the viral Kcv channels can be used as building blocks in artificial nanosized biosensors, i.e. devices composed of a biological and a physical/chemical part that are able to detect chemical and physical parameters and transform them into electrical signals.

The viral K\(^+\) channels are very suitable for protein bioengineering due to their small size and their similarity to the known crystal structure of K\(^+\) channels. These features also make them ideal candidates for computational approaches to understand their function and for \textit{in silico} protein design. Atomic scale X-ray crystallography which is the basis for modelling proteins with MD simulations revealed the structure of the bacterial channel KcsA (Doyle \textit{et al.} 1998). In contrast to KcsA and other channel models, a unique feature of the Kcv model is its functionality in the sense that it allows one to study spontaneous ion translocations over the entire pore in a reasonable simulation time without any artificial, biasing intervention (Tayefeh \textit{et al.} 2007, 2009). This system forms the basis for the prediction of I/V curves based on molecular information, i.e. a quantitative descriptor of functionality. Applied evolutionary optimization strategy allows for the rational design of particular desired features of the channel protein. An alternative strategy to identify channels with interesting functional properties is mining for new viral protein candidates in aquatic systems. It is known that 1 ml of coastal water can contain as many as \(10^8\) viruses of many different species (Suttle 2005; Corneau \textit{et al.} 2006), offering the potential of an enormous source of channels with diverse structures and functions. To this end, the viral Kcv channels are a promising tool for bioengineering and computational studies.
end, exemplary cloning and sequencing of K\textsuperscript{+} channel genes from 40 Paramecium bursaria chlorella virus species (PBCV-1) revealed a remarkable diversity with respect to structure and function of the viral channels (Kang et al. 2004). Thus, both rational protein engineering and gene mining are promising strategies to identify proteins with new structures and functional features with potential for future development in protein-based nanodevices.

3. Conclusions and outlook

Biopolymers such as proteins have evolved over millions of years and perform a multitude of specific functions, which often exceed man-made materials in their properties. The most attractive argument to consider biomimetics as a tool for the development of innovative technologies is its potential of a broader or even deeper impact that may not be restricted to a certain type of technology or field of application. This paper has provided several concepts for molecular biomimetics of proteins: spider silk proteins can be used as structural fibres, or films and nanocapsules for functionalized biomaterials in regenerative therapy, while AFPs, Mefp or channel proteins may be used for specifically functionalized surface coatings or nano(bio)technological biosensors and molecular switches by protein engineering, respectively. Thus, proteins have an important role in future molecular biomimetic systems, and nature could serve as a ‘think tank’ for innovative material science and nanotechnology. Future work will unquestionably focus on technical implementation, and proving the marketability of such new technologies.

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