Antheraea pernyi Silk: Mechanical and Thermal Properties

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Deakin University
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I am the author of the thesis entitled

*Antheraea pernyi* Silk: Mechanical and Thermal Properties

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Refereed journals


To be submitted

1) The effects of crystalline structure on the physical properties of thermally annealed silk fibres.

2) Thermal conductivity of single *A. pernyi* silk fibres

Conference proceedings

Contributions to Other Publications during this study


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Abbreviations

AF – *Antheraea pernyi* fibroin
AFM – Atomic Force Microscopy
*A. pernyi – Antheraea pernyi*
AS – *Antheraea pernyi* sericin
ATR – Attenuated total reflectance
A/Ala – Alanine
*B. mori – Bombyx mori*
BF – *Bombyx mori* fibroin
BS – *Bombyx mori* sericin
CA – Contact angle
DI water – Deionised water
EDTA – Ethylenediamine tetraacetic acid
EDX – Energy dispersive X-ray spectroscopy
FTIR – Fourier transform Infrared spectroscopy
G/Gly – Glycine
IGC – Inverse Gas Chromatography
LiSCN – Lithium Thiocyanate
MFP – Phonon mean free path
Na₂CO₃ – Sodium carbonate
NaHCO₃ – Sodium bicarbonate
PEE/PPy – Pentaerythritol/polypyrrole
QCM-D – Quartz Crystal Microbalance with Dissipation
SEM – Scanning electron microscopy
SDS-PAGE – Sodium dodecyl sulfate polyacrylamide gel electrophoresis

S/Ser – Serine

UV – Ultraviolet

XRD – X-ray Diffraction
Introduction

Research background

Silkworm silk fibres have been studied for decades. Silk can be spun by several species of arthropods, the most significant of which are *Bombyx mori* (*B. mori*) and *Antheraea pernyi* (*A. pernyi*). A single silk fibre is secreted from two spinnerets of a silkworm with two fibroin cores glued by a sericin coating. By moving its head, a silkworm spins silk fibres to construct a silk cocoon from the inside out. The primary function of a cocoon is to protect pupa during metamorphosis until the formation of an adult moth. Unlike the domestic *B. mori* cocoon, the wild *A. pernyi* cocoon has a peduncle to connect to twigs and it can protect the worm in a harsh environment.

Various silkworm cocoons can be unravelled as continuous threads, permitting a much stronger cloth to be woven from silk fibres. Silk fabrics were first developed in ancient China dating back to 3630 BC. Compared to the *B. mori* silk, the *A. pernyi* silk has been developed much later, and is not as popular as *B. mori*, due to its smaller production, difficulties in fibre unravelling and dyeing, shorter fibre length and inferior uniformity in colour and texture. However, *A. pernyi* has been found to have excellent tensile strength and extensibility and is biocompatible [1]. It has been transformed from traditional textiles into high-technology fields, including tissue engineering [2, 3] and delivery vehicles for therapeutic agents [4]. Apart from the tripeptide sequence Arg-Gly-Asp known for cell attachment [1], *A. pernyi* fibres have outstanding mechanical properties, which are determined by a combination of the primary sequences and the secondary structure of protein. This has inspired considerable
research on the artificial spinning of *A. pernyi* silk fibres. It has been found that by changing the reeling speeds [5], hot-stretching [6] and UV irradiation [7], the mechanical properties of the silk fibres can be manipulated.

Despite the significant research interest in *A. pernyi* silk for high performance materials, a full understanding of differences in fibres from the different parts, namely peduncle, outer floss and the cocoon shell, of the *A. pernyi* cocoon has not been achieved. All these parts contribute to the protective function (e.g. thermal regulation) of the cocoon, especially from natural predators and in harsh environments (e.g. extreme temperatures, -40 and 40 °C). The physical properties of natural protein fibres are affected by their crystalline structure [8, 9]. To understand the super mechanical and thermal regulation properties of the *A. pernyi* cocoon, it is essential to investigate the properties of the individual fibres from the different parts of the cocoon. To this end, the structural differences in the fibres on different scales (microscopic and nanocrystalline) and their effects on the properties of the fibres have to be revealed. This would also help the design of biomimetic materials. Despite the excellent properties of the *A. pernyi* silk, its applications have been limited by the difficulties in degumming. The development of an efficient degumming method needs a fundamental understanding of the adhesion between fibroin and sericin from this wild silk.

**Research objectives**

This project aims to understand the correlations between the fibre structures, mechanical and thermal properties of silk fibres from the different parts of the *A. pernyi* cocoon, as well as the adhesion between fibroin and sericin from *A. pernyi*. To this end, *B. mori* silk fibres are used for comparison and other natural fibres including
ramie, wool and cotton are also included in the evaluation of thermal properties. In summary, this thesis covers the following four parts.

- Firstly, structure and mechanical properties of fibres from the different parts of *A. pernyi*, i.e. peduncle, outer floss, outermost and innermost fibres, are investigated to understand if the fibre structure can be related with the protection provided by this wild silkworm cocoon.

- Secondly, the nanocrystallite structure versus temperature is examined to elaborate how it affects mechanical properties, by a comprehensive comparison of the structures of fibres from different parts of the *A. pernyi* cocoon and *B. mori* silkworm silk fibres.

- Thirdly, the thermal conduction properties of fibres from different parts of the *A. pernyi* cocoon, *B. mori* cocoon and some other natural fibres, in both the longitudinal and transverse directions are studied. The crystalline structure of the silk fibres are tuned by thermal annealing to understand how the crystalline structure affects the thermal conduction of the fibres.

- Finally, the adhesion properties between fibroin and sericin from *A. pernyi* and *B. mori* fibres are investigated to understand the difficulties in degumming *A. pernyi*. 
Abstract

This project reveals the differences in the structure, mechanical and thermal properties of silk fibres from different parts of the *A. pernyi* cocoon, the influence of the nanocrystalline structure on the properties, and the adhesion between silk fibroin and sericin proteins.

Firstly, the raw silk fibres from different parts of *A. pernyi* cocoon, including peduncle, outer floss, outermost fibre and innermost fibre, were studied to understand their structures and properties. The outermost fibre and outer floss fibre have relatively high contents of sericin, whereas more pores were found in the cross-sections of fibres from peduncle and outer floss fibres. The outermost fibre showed superior abrasion resistance, mainly due to the presence of mineral crystals. Studies on tensile strength and nanohardness on longitudinal and transverse directions of fibres demonstrated that peduncle fibres had the lowest elasticity and hardness in transverse direction but relatively high modulus in longitudinal direction, indicating good plastic deformation which is consistent with the results of Dynamic Mechanical Analyser (DMA). Fourier Transform Infrared Spectroscopy (FTIR) and X-Ray Diffraction (XRD) were used to identify the correlation between secondary structure and the mechanical performance. It revealed that the high content of intermolecular β-sheets and random coil of outermost fibres may account for their superior fracture strength and extensibility.

Secondly, the nanocrystalline structure of silk fibres was tuned by thermal treatment in order to understand how the structure affects the mechanical properties of fibres. It has been found that, along with an increase in temperature for thermal treatment, the overall content of β-sheet, a sum of intermolecular and intramolecular β-sheet, was
increased, whereas that of intermolecular β-sheet decreased leading to progressive reduction in the breaking tensile strength. In contrast, the increasing intramolecular β-sheet has a positive effect on the elastic modulus and hardness. The yield strength is proportional to the average crystallite size which increases along with the annealing temperature.

Thirdly, the thermal conduction of raw silk layers was investigated via Laser flash (LFA). It demonstrated that the innermost layer has a higher thermal conduction than the outermost layer, and the main contributor to thermal conduction of raw silk cocoon is calcium oxalate crystals followed by sericin layer. The thermal conduction of single fibres was also studied. It was observed that thermal conduction exhibited strong anisotropy in transverse and longitudinal directions, with higher thermal conductivity in the longitudinal direction. Thermal conduction in longitudinal direction is affected by crystallite size. The crystallites size in the directions of β-sheet stacking and β-strand direction had a tendency to increase the thermal conduction of *A. pernyi* outermost fibres, whereas increasing β-sheet stacking and the number of chains can increase thermal conduction of *A. pernyi* innermost fibres. The high mean free path of phonon from innermost fibre promoted its high thermal transfer in longitudinal direction. The nano pores inside *A. pernyi* silk fibres are enclosed pores, and have a negative effect on the heat transfer in the longitudinal direction due to the high inclination degree of the nanocrystallites. With the inclination degree of 90°, the average thermal transfer was attributable to the hydrogen bonds instead of chain direction.

Finally, the adhesion between sericin and fibroin was investigated to illustrate the reasons for the difficult degumming of *A. pernyi* fibres. In this study, Inverse Gas
Chromatography (IGC) was performed to understand the adhesion between fibroin and sericin of fibres from the different parts of the *A. pernyi* cocoon. It was found that the adhesion is mainly specific adhesion with a high degree of heterogeneity. FTIR and nuclear magnetic resonance (NMR) studies indicate that the specific adhesion is attributable to the intermolecular hydrogen bonding arising from the glycine from fibroin and serine from sericin. Quartz Crystal Microbalance with Dissipation (QCM-D) and contact angle (CA) measurements showed that for *A. pernyi*, the adsorption of sericin on fibroin was faster than the adsorption of the proteins from *B. mori*.

**Significance**

- The understanding of fibres from different parts of the *A. pernyi* cocoon as well as *B. mori* cocoon fibres, provides guidance to design of material with desirable properties for different applications. Meanwhile, it may also promote the use of silk fibres from peduncle and outer floss of the *A. pernyi* cocoon, which are mostly discarded during silk processing.

- This work demonstrates that the mechanical properties of silk fibres can be effectively tuned by changing the crystalline structure via thermal annealing. This offers a convenient approach to manipulate the mechanical properties of silk fibres for various applications.

- Characterising the thermal conduction of fibres particularly in the transverse direction has remained a challenge. The method developed here can be generally used for other types of fibres. Correlation between the thermal property and the crystallite can improve our fundamental understanding of the nanoscale thermal transport phenomena in natural fibres. This may further guide the design of synthetic silk materials with tuneable thermal properties.
The strong adhesion between fibroin and sericin could inspire the design of high performing composite materials with excellent interfacial adhesion between the different components.
CHAPTER 1 Literature Review

1.1 Introduction

1.1.1 Categories of silk cocoons

Silk fibres spun by several species of arthropods have existed for thousands of years. The categories that have attracted significant interest are spider silk and silkworm silk. Spider silk is produced to form orb-weaving web for catching and wrapping its prey; whilst silkworm silk is produced to form a cocoon that protects a pupa from harsh environments and physical attacks. Due to their smooth texture, lustre and strength, as well as feasibility of mass production, silks from silkworms have been extensively used in apparel and fashion applications for thousands of years.

As early as the Han Dynasty (around 130 BC) in China, there had been the Silk Road which accelerated sericulture exchange between the midland and western regions. This silk is mostly called silkworm silk spun by silk moths. The families of silk moths have been growing and branching into several small groups mainly according to their regions of origin (Figure 1.1). Commercial silks are produced by larvae of moths with diverse varieties. These species include at least four families: Bombycidae, Saturniidae, Lasiocampidae, Notodontidae (Figure 1.1) [10]. Bombyx mori (B. mori), the most important domestically reared, has a history of almost four thousand years in China. B. mori silk is easy to unwind from cocoon and reel into fibres. While for the Saturniidae genera, a kind of wild silkmoth, there are mainly three families which are Samia, Antheraea and Attacus. Antheraea family is well known for its sub-branches including Antheraea pernyi (A. pernyi, Chinese oak tasar silkmoth), Antheraea mylitta
(A. myllita, Indian tropical tasar silkworm), Antheraea assamensis (A. assamensis or Muga, Indian coarse golden brown or amber semi-domesticated silkworm), Antheraea yamamai (A. yamamai, Japanese oak silkworm), and Samia cynthia ricini (Eri, Indian castor silkworm) [11].

**Figure 1.1** Schematic diagram of silk families [10, 11]

**Figure 1.2** shows various silk species.
Figure 1.2 The silk species. (a) Spider silk; (b) B. mori silkworm; (c) A. pernyi silkworm; (d) A. mylitta silkworm; (e) Muga; (f) Eri; (g) A. yamamai silkworm.

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1.1.2 Natural silk processing and the formation of silk cocoons

A cocoon is woven from the inside out, at the end of the larval stage of a silkworm. Figure 1.3 shows the formation process of a cocoon.

Figure 1.3 The process for silk cocoon spinning [12]

A single silk fibre is produced from glands in a silkworm and each of the glands is comprised of posterior and middle regions and an outlet for silk spinning. A silk fibre is a core-shell structure. The core, produced in the posterior region is made of fibroin,
a type of silk protein that is stored in the glands as an aqueous solution. Another kind of protein in the silk fibre is sericin, which is produced from the middle region of the gland and, forms a non-uniform sticky coating for the core fibroin filaments [13, 14]. Unlike its counterpart B. mori, A. pernyi fibroin dope has many pores that are produced by vesicles in its posterior region [15]. These pores are elongated during spinning through the spinneret and persist in the silk fibres.

**Figure 1.4** The anatomical diagrams of the A. pernyi (a) and B. mori (b) silk cocoon

A domestic silkworm cocoon such as B. mori, consists of a cocoon shell and outer floss; whilst wild silkworm cocoon, such as that of the A. pernyi, is composed of peduncle, outer floss and the cocoon shell (See **Figure 1.4**), which account for 2.22%, 11.55% and 86.22% of the whole weight, respectively. Peduncle, which is absent in B. mori, connects the A. pernyi cocoon to twigs and has a very high tensile strength, up to 94 MPa [16].

1.2  A. pernyi silk cocoons studied in this project

This project mainly focused on silk produced by A. pernyi silkworm, which is known as Chinese tasar silkworm and feeds on leaves of Chinese oak trees. It is a major source of wild silk fibres. The annual production of A. pernyi silkworm cocoons is about 50,
000 tons [17]. However, peduncle and outer floss, which are minor parts of the *A. pernyi* cocoon, are mostly discarded in silk processing. Little research had been done on understanding the differences of fibres from these three parts: peduncle, outer floss and the cocoon shell, which is the focus of this project.

1.2.1 Morphology and structure of the *A. pernyi* cocoon

The morphology and structure of a cocoon are essential for a silkworm to survive. The type, size, shape and colour of the cocoons are specific to species and show considerable variations. Generally, wild cocoons hang on the host plant (such as *A. pernyi*), are attached to the host plant (*Samia*), spun among leaves without being attached (*Actia Luna*) [18]. In terms of construction of the cocoons, silkworms such as *cecropia* produce cocoons with three distinct layers whereas *R. lebeau* and other silkworms produce cocoons with a single layer of fibres. Similarly, *Saturniidae* produce compact and solid cocoons whereas *Copaxa multifenestrata* produce compact cocoons with perforations that allow air circulation [19]. Also, it is reported that large cocoons are prone to have unique properties [18].

![Figure 1.5](image-url)

**Figure 1.5** microstructures of silk fibres produced by silkworm and spiders (a) [20] and hierarchical structure of silkworm silk fibre and spider silk fibre (b) [21] and fibril in silkworm *B. mori* silk and spider silk (c) [22]
A wild *A. pernyi* silkworm, similar to *B. mori* and other silkworms, has two spinnerets to spin two fibroin filaments simultaneously [18, 23]. The core fibroin filaments are conglutinated together by amorphous silk protein gum (sericin) [24, 25]. Spider silk shows a skin-core globular microstructure (See Figure 1.5). The fibroin or spidroin fibre is made of bundles of fibrils oriented along the fibre axis [26], the widths of which however, are independent of the fibre sizes [27]. *Nephila clavata* dragline consists of microfibrils with a diameter of about 100 nm [28]. Silks from *B. mori* (136 nm), *A. pernyi* (109 nm) and *A. yamamai* (110 nm) have the geometric mean fibril widths above 100 nm [27].

Like many biological materials, silk features a hierarchical structure arising from a complex assembly process. These fibrils are extruded and shear flowed to increase alignment and connectivity by protein structures, which have many strands of a single repetitive protein sequence [29].

1.2.2 Amino acids composition of *A. pernyi* and *B. mori* silks (primary structure)

Silk fibrils are assembled by fibroin proteins, which have highly repeated amino acid sequences in the middle and non-repetitive residues on the ends. The repetitive regions largely determine the macroscopic, such as mechanical properties of the fibrils, whilst fibre elongation mainly depends on non-repetitive terminal domains which can be post-translationally modified and proteolytically processed [30]. Silk fibroin consists of 18 different amino acids. The main chains of *B. mori* are made up of glycine and alanine with occasional substitution by tyrosine and valine, et al. In contrast, *A. pernyi* fibroin contains primarily alanine. The typical amino acids of fibroin proteins from these two silkworm silk and spider dragline silk are shown in Figure 1.6.
Spideroins consist of a large repetitive core domain interconnected by non-repetitive amino and carboxy terminal domains [30]. Silkworm fibroin consists of a covalently linked, highly repetitive heavy chains and non-repetitive light chains. The silk fibroin from *B. mori* consists primarily of glycine, alanine, and serine residues in a molar ratio of 3:2:1. Glycine and alanine form typical $-\text{(ala–gly–)}_n-$ repeating motifs [32]. Compared to its domestic counterpart *B. mori*, *A. pernyi* silk has more sequences similar to those of spider dragline silk (See Figure 1.6). Poly $-(\text{ala})_n-$ sequences mainly make up the crystalline regions of *A. pernyi* and *Nephila clavipes* dragline silk [33]. Sericin from silkworm silk has high contents of serine and charged amino acid residues, especially Glu and Tyr [15], with *A. pernyi* sericin having a lower percentage of serine and tyrosine than that of *B. mori* sericin [34]. Table 1.1 shows the typical compositions of fibroin and sericin from *B. mori* and *A. pernyi*, respectively. The considerable difference in their chemical composition would lead to various physical properties.
Table 1.1 Amino acids composition (mol %) of *B. mori* and *A. pernyi* silk [16, 35-39]

<table>
<thead>
<tr>
<th>Amino acids (mol/%)</th>
<th><em>A. pernyi</em> silk fibre</th>
<th><em>A. pernyi</em> Sericin</th>
<th><em>B. mori</em> Fibroin</th>
<th><em>B. mori</em> Sericin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>80-85%</td>
<td>15-20%</td>
<td>70-75%</td>
<td>25-30%</td>
</tr>
<tr>
<td>Alanine (Ala)</td>
<td>50.5</td>
<td>2.82</td>
<td>30.0</td>
<td>5.5</td>
</tr>
<tr>
<td>Glycine (Gly)</td>
<td>23.6</td>
<td>12.55</td>
<td>42.9</td>
<td>14.30</td>
</tr>
<tr>
<td>Serine (Ser)*</td>
<td>11.3</td>
<td>23.49</td>
<td>12.2</td>
<td>39.0</td>
</tr>
<tr>
<td>Tyrosine (Tyr)*</td>
<td>8.8</td>
<td>5.64</td>
<td>4.8</td>
<td>0.70</td>
</tr>
<tr>
<td>Aspartic (Asp)*</td>
<td>6.58</td>
<td>17.72</td>
<td>1.9</td>
<td>13.3</td>
</tr>
<tr>
<td>Arginine (Arg)*</td>
<td>6.06</td>
<td>5.49</td>
<td>0.5</td>
<td>2.90</td>
</tr>
<tr>
<td>Threonine (Thr)*</td>
<td>0.69</td>
<td>15.26</td>
<td>0.9</td>
<td>3.30</td>
</tr>
<tr>
<td>Glutamic acid (Glu)*</td>
<td>1.34</td>
<td>6.86</td>
<td>1.4</td>
<td>12.80</td>
</tr>
<tr>
<td>Proline (Pro)</td>
<td>0.44</td>
<td>1.63</td>
<td>0.5</td>
<td>Nd</td>
</tr>
<tr>
<td>Cysteine (Cys)</td>
<td>0.04</td>
<td>0.27</td>
<td>0.0</td>
<td>Nd</td>
</tr>
<tr>
<td>Valine (Val)</td>
<td>0.95</td>
<td>1.56</td>
<td>2.5</td>
<td>0.70</td>
</tr>
<tr>
<td>Isoleucine (Ile)</td>
<td>0.69</td>
<td>0.60</td>
<td>0.6</td>
<td>0.20</td>
</tr>
<tr>
<td>Leucine (Leu)</td>
<td>0.51</td>
<td>0.69</td>
<td>0.6</td>
<td>0.50</td>
</tr>
<tr>
<td>Phenylalanine (Phe)</td>
<td>0.52</td>
<td>0.99</td>
<td>0.7</td>
<td>Nd</td>
</tr>
<tr>
<td>Lysine (Lys)*</td>
<td>0.26</td>
<td>1.61</td>
<td>0.4</td>
<td>5.40</td>
</tr>
<tr>
<td>Histidine (His)*</td>
<td>1.41</td>
<td>1.70</td>
<td>0.2</td>
<td>1.0</td>
</tr>
<tr>
<td>Total polar amino acid</td>
<td>36.1</td>
<td>77.77</td>
<td>22.3</td>
<td>78.4</td>
</tr>
</tbody>
</table>

Note: The asterisk indicates that is polar amino acid.

‘(+)+’ indicates that is positively charged amino acid.

‘(-)-’ indicates that is negatively charged amino acid.

1.2.3 Protein structure

*B. mori* silk is composed of heavy chain fibroin (H-fibroin, 250-500 KDa) and light-chain fibroin (L-fibroin, 25 kDa), which are covalently linked in a 1:1 molar ratio through a single disulphide bridge [32]. In addition, Glycoprotein, p25 (30kDa), is associated with the H-L complex by non-covalent interactions [40]. The molar ratio of
the three components is 6:6:1 [24, 41]. These proteins are coated with sericin (~180 kDa) that are hydrophilic [42, 43]. In contrast, the fibroin protein of wild silkworm silk has the H-H structure, indicating that the main component is made of heavy chain fibroin [44], with a molecular weight of 400-450 kDa [45]. In addition, it was reported that *A. pernyi* fibroin has a polypeptide with 261 amino acid residues and eight cysteine residues paired in four disulphide bridges [46]. The molecular weight of *A. pernyi* sericin ranges between 10 and 310 kDa [47, 48].

Silk sericin proteins can be obtained from silk fibres by degumming and fibroin proteins can then be regenerated by dissolving the degummed silk fibroin fibres with suitable solvents. The proteins obtained from silk fibres are normally degraded during degumming and dissolution of fibroin fibres. Degumming especially with harsh solvents such as alkaline (e.g. Na₂CO₃) solutions can cause serious breaking down of silk proteins. The damage is particularly serious for sericin proteins, for example, the sericin obtained from *B. mori* silk has a molecular weight of less than 20 or 5 kDa [49], which is significantly lower than that of the natural sericin from this type of silk. Degumming with alkaline solutions can also cause degradation of *B. mori* fibroin peptide strands into fragments with molecular weights between 50 and 200 kDa [50]. Urea is a mild degumming agent. The molecular weight bands of regenerated *B. mori* fibroin are very similar to its natural state when urea was used. For *A. pernyi* silk, stronger reagents such as Ca(NO₃)₂ and LiSCN have to be used to dissolve fibroin fibres [45]. These reagents cause much more serious damage to *A. pernyi* fibroin proteins than the alkaline degumming solutions.
1.2.4 Hierarchical structure (secondary and tertiary structure)

The heavy chains are packed together by a close network of strong interplays (hydrogen bonds) between amide linkages of the adjacent chains. β-sheets crystals of *A. pernyi* silk is mainly composed of repetitive stretches of polyalanine [51] (See Figure 1.6). Results from solid NMR indicates that the crystals consists of antiparallel and parallel β-sheets roughly at a ratio of 2:1 [52]. The molecular geometry of the β-sheets nanocrystals is defined by strands of a single, repetitive protein sequence [53]. The crystalline region of fibroin is mostly hydrophobic and plays an important role in determining the physical properties, biodegradability and biocompatibility of a fibroin fibre.

As the secondary structure becomes established based on primary structure, a polypeptide folds and refolds upon itself to assume a complex three-dimensional shape called the protein tertiary structure. The formation of a tertiary structure is due to intramolecular interactions (covalent bonds, hydrogen bonds, ionic bonds, and van der Waals interactions) between the functional side groups along the polypeptide chain. The intramolecular interactions and intermolecular interactions together stabilise a protein’s overall three-dimensional conformation. Figure 1.7(a) is a XRD pattern of *B. mori* fibroin fibre, showing orderly aligned β-sheet structures along the axis of the silk fibres. As shown in Figure 1.7(b) and (c), the β-sheet crystallites consist of anti-parallel arrangement (*i.e.*, adjacent β-strand run in the opposite direction), rather than parallel arrangement (*i.e.*, adjacent β-strand run in the same direction). The intermolecular hydrogen bonds exist in the forms of longer non-linear hydrogen bonds (2.97 Å) between parallel β-strands and shorter linear hydrogen bonds (2.76 Å)
between antiparallel β-strands. The intramolecular hydrogen bonds mainly occur along the peptide chains and the residual amino acids.

**Figure 1.7** XRD pattern of *B. mori* silk fibroin fibre (a) [54]; bond length and bond angle in parallel and antiparallel fibroin β-sheets (b) [55, 56]; widely accepted polar-stacking of antiparallel β-sheets (c) [54, 55]

### 1.2.5 Crystalline structure

An understanding of the crystal structure of natural silk is essential for producing biomimetic silk fibres having the unique properties of their natural counterparts. The crystalline nature of silk fibres has been investigated primarily by means of XRD to
illustrate $\beta$-sheet models [54]. The large twisted crystals of $B. \text{ mori}$ silk are made up of smaller chain-folder, lamellar crystals approximately 5 nm in thickness [57]. Dark field TEM revealed that the sizes of the $\beta$-sheet crystallites in $B. \text{ mori}$ silk ranged from 20-170 nm and from 1 to 24 nm, in longitudinal and lateral directions, respectively [58]. $Nephila \text{ clavata}$ dragline consists of microfibrils with a diameter of about 100 nm, which is subdivided to amorphous and crystalline regions with size 20-50 nm without any characteristic markings [28].

Lattice index is the basic structural parameters of crystal material, which is directly related to the binding energy between atoms. The variations of lattice index is a reflection of the changes of the components inside the crystal and the force loading conditions. The amino acid sequence of the crystalline region of $B. \text{ mori}$ is GAGAGS, and its lattice index parameters can be shown as $a = 0.938 \text{ nm}$, $b = 0.949 \text{ nm}$ and $c = 0.698 \text{ nm}$ in a monoclinic space group ($c$-axis parallel to the fibre axis and $a$ and $b$ axes normal to and randomly distributed about the fibre axis) [59]. Small crystallite size (tens of nanometer) is not only consistent in $B. \text{ mori}$ fibres, but also in other silks, like $A. \text{ mylitta}$ ($a = 1.06 \text{ nm}$, $b = 0.944$, $c = 0.695$), and $Nephila$ silk ($a = 1.57 \text{ nm}$, $b = 0.944 \text{ nm}$, $c = 0.695 \text{ nm}$) [60]. It is expected that the mechanical and thermal properties of silk fibres critically depend on their crystallite size, aspect ratio and size distribution. The crystallite size of silk fibres from different parts of $A. \text{ pernyi}$ have not been investigated, and the correlation between the crystalline structure and the mechanical as well as thermal properties have not been understood as well.

1.2.6 Crystalline structure versus mechanical and thermal properties

The unique combination of high tensile strength and high extensibility have contributed to significant research interest in silk fibres. The stiffness and the strength
of a silk fibre are dictated mainly by its β-sheet crystallites, where the covalent bonds, interchain hydrogen bonds, together with intersheet van der Waals and hydrophobic interactions contribute significantly to the stability of the structure. Fundamentally, silk fibroin can form intramolecular/intermolecular β-sheet crystallites, parallel/antiparallel β-sheet as well as β-sheet crystallites of different sizes and orientations along fibre axis.

A β-sheet consists of extended polypeptide strands (β-strands) connected laterally by at least two or three backbone hydrogen bonds, forming a generally twisted and pleated sheet. Both silkworm and spider silk contain β-sheets with anti-parallel strands (known as crystallite). The intermolecular hydrogen bonding exists in the anti-parallel β-sheet whereas the intramolecular hydrogen bonding exists along polypeptide chain in amorphous region [61]. The anti-parallel β-sheet contains individual protein chains aligned side-by-side with every other protein chain aligned in the opposite direction. The strands aligned with the fibre axis make a parallel-β structure [62], whereas the strands perpendicular to the fibril axis form a cross-β structure [63]. These two structures could be identified by the orientation function, i.e. alignment of the strands with respect to the axis. They can also be calculated by the degree of crystallinity. Silk also contains a type of parallel β-sheet which refers to the relative (parallel) orientation of adjacent strands [64]. Figure 1.8 shows the diagrams of different configurations.
1.2.7 Extraction of calcium oxalate crystals from wild silkworms

Different from domestic silkworm cocoons, cocoons of wild silkworms, such as, *A. assama* [38], *A. pernyi*, *S. cynthia* and *A.mylitta* [65] usually have crystals on their outer layers as shown in Figure 1.9. It is reported that these mineral crystals are a kind of hindgut exudate, left by the silkworm during spinning (excrements) [38, 66, 67]. They contain a substantial amount of calcium oxalate crystals, which is extremely...
interesting. These crystals tan the wild silk fibres [66], and affect the thermal [68], mechanical [69, 70] and optical [71], adsorption of proteins [72, 73] and UV protection of wild silkworm cocoons [71].

Figure 1.9 SEM photographs of wild silk cocoon shells with mineral crystals. (a) *S. Cynthia*; (b) *A. pernyi*; (c) *A. mylitta*; [65] (d) *A. assama* [38].

To isolate these crystals, physical [74] and chemical methods [75] can be applied. They can also be removed in the degumming process, which also remove sericin at the same time [38].

1.2.8 Extraction of sericin

Sericin is hydrophilic global protein synthesized exclusively in the middle silk glands of silkworm. It constitutes 20~30 wt% for *B. mori* [76] and 5~12 wt% for *A. pernyi* [77], of the total cocoon weight. Sericin from *B. mori* has many excellent performances including oxidation resistance [78], anti-bacterial effect, UV resistance [35],
biodegradation and promotion of cell proliferation [79]. Sericin from non-mulberry, like *A. pernyi*, has been proven by Kundu *et al.* to have the ability of inhibiting UVB [80] and hydrogen peroxide-induced cell death [81]. These different physical properties of *B. mori* and *A. pernyi* sericin should be originated from their different compositions of amino acids. It is reported that the high hydrophilicity and hence absorbency and release of moisture of sericin is attributed to the high content of polar amino acids, such as serine and aspartic acid [35].

For applications, sericin is usually extracted to get protein fibres, sponges, films and gels. It is extracted during the degumming process of silk fibres, which involves the cleavage of the peptide bonds of sericin, either by hydrolytic or enzymatic methods. As indicated by Table 1.2, *B. mori* silk fibre can be easily degummed with neutral water. In contrast to *B. mori*, *A. pernyi* silk fibres, due to its small amounts of silk sericin, should be able to be degummed easily. However, the degumming agents for *A. pernyi* are normally more intense than that for *B. mori* silk cocoon [75, 82, 83]. This indicates that the adhesion between fibroin and sericin from *A. pernyi* silk shall be stronger. So far, the adhesion properties between silk fibroin and silk sericin from domestic and wild silkworm cocoons have not been investigated.
Table 1.2 Degumming methods for *A. pernyi* and *B. mori* silk cocoons

<table>
<thead>
<tr>
<th>Silk cocoons</th>
<th>Degumming agents</th>
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</thead>
<tbody>
<tr>
<td><em>A. pernyi</em></td>
<td>Alcalase and NaHCO$_3$ 5g/L [83]; 0.5% (or 5 g/L) Na$_2$CO$_3$ for two (or three) 30 min changes [36, 84]; 0.3% soap (1000 mL) plus 5 g of Na$_2$CO$_3$ at 98 °C for 30 min [85]; 2% base solution (Na$_2$CO$_3$ plus NaHCO$_3$ in equal weight proportion) for 1 h [71];</td>
</tr>
<tr>
<td><em>B. mori</em></td>
<td>0.5 % Na$_2$CO$_3$ for 1h [50, 75]; soap-soda method for 30 min [27, 86]; base 2% Na$_2$CO$_3$ plus 2% NaHCO$_3$ for 30 min [87]; 0.02 M Na$_2$CO$_3$ for 30min [88, 89]; Ultrapure water at 105 °C or 120 °C for 1h [90]; 1% sodium hydrogen carbonate [91]; Distilled water for 30 min [92]; Boiling in a 0.7% soap solution for 1 h [93];</td>
</tr>
</tbody>
</table>

1.2.9 Fibroin fibres

*A. pernyi* silk fibroin filaments show the presence of distinct longitudinal striations, with a thickness of 0.4–1 μm. This characteristic is common to other wild silk filaments [38], which is a striking contrast to the very smooth appearance of *B. mori* silk filaments, and accounts for the lower degree of luster of textile products made by wild silk. It is worth mentioning that the average diameter of a wild fibroin filament is
twice that of *B. mori* [38]. Fibre cross sections of wild silk filaments are variously shaped with the elliptical shape being prevalent (See Figure 1.10). Voids are visible within the cross section of wild silk filaments, while absent in that of *B. mori* silk. However, the fibre porosity and properties of pore like pore size and density, have not been investigated, for *A. pernyi* silk.

![Figure 1.10 SEM photographs of the cross sectional areas of *B. mori* (a) [94], *A. assama* (b) [38], *A. pernyi* (c) [95] and *A. mylitta* (d) silk fibres](http://jswsmo.appspot.com/randde.html)

As mentioned above, the secondary structure of the silk fibroin fibres contributes to their unique properties, such as high strength and elasticity. These properties can further be affected by ions (such as Fe$^{3+}$, Zn$^{2+}$ and Ca$^{2+}$) [96, 97], surfactants [98],
1.3 Mechanical properties of *Antheraea pernyi* silk cocoon and fibres

1.3.1 The cocoon

The *A. pernyi* silk cocoon has higher modulus and hardness than *B. mori* in the thickness direction [69]. Moreover, the tensile strength is much higher in the longitudinal direction than in the transverse direction [103, 104]. The *A. pernyi* cocoon and *B. mori* cocoon both exhibit a graded layer structure. Their mechanical strength increases from the outer to the inner layers, due to the gradual decrease of porosity, decrease of sericin content and increase in fibre density in that direction [105].

1.3.2 Raw silk fibres and degummed silk fibres

A correlation between the mechanical properties of a cocoon and the individual fibres that construct the cocoon is hard to establish [70], since a composite, the mechanical properties of a cocoon are also contributed by many other factors such as the adhesion between fibres. As the unique feature of wild silk cocoons, like *A. pernyi*, peduncle has a high tensile strength around 94 MPa so that a force approximately of 200 N is needed to break it [16]. However, the tensile strength of single peduncle fibres has not been studied so far. Understanding the mechanical properties of single fibres from peduncle and other parts of the *A. pernyi* cocoon is significant for tailored design of fibres for different applications and for promoting the use of silk fibres that are generally discarded during silk processing.

It is widely accepted that silk degumming can affect the tensile strength of silk fibres [94]. The strength of silk fibres can be well maintained under mild degumming
conditions, whereas aggressive degumming conditions result in significantly weaker strength. Compared to strength, elongation is less affected [13].

1.3.3 Breaking mechanism

*A. pernyi* silk proteins contain highly oriented alanine-rich nanocrystals of \( \beta \)-pleated sheets embedded in a glycine-rich matrix of random polypeptide chains along the fibre axis [106] and moderately oriented helical structures [107]. The intermolecular hydrogen bonds connect polypeptides along the interchain direction to form \( \beta \)-sheets in crystallites, whereas the intramolecular hydrogen bonds connect a single polypeptide to form \( \beta \)-sheets embedded in a glycine matrix.

The extensibility of a silk fibre is sacrificed by hydrogen bond (H-bond) network breaking and the strength lies on the stretching of covalent bonds along the protein backbone (shown in Figure 1.11). At small tensile strains, the breakage of intrachain H-bond in the glycine-rich matrix is dominant and the alignment of nanocrystals is slightly improved. When the strain is progressively increased, the breakage of interchain H-bond in crystalline-pleated sheets and the stretching of covalent bonds along the protein backbone start to play a dominant role. The alignment of both the inter- and intra- molecular \( \beta \)-sheets are strongly enhanced [106]. Figure 1.11 shows the stress-strain curve of *N. pilipes* dragline fibre which has repetitive peptides very similar to that of *A. pernyi* silk.
1.3.4 Crystalline structure versus mechanical properties

The differences in mechanical properties of various silk fibres are due to structural differences derived from different amino acid compositions and sequences [4]. Even though long poly (alanine) sequences contribute to β-sheet crystals, the poly (alanine) region is partially crystallised [108]. In antiparallel β-sheet crystallites, the protein chains are held together by intermolecular hydrogen bonding between amide groups of two separate chains. The splitting of intermolecular β-sheet crystallites can lead to failure of the material.

Molecular modelling and simulation have been performed to gain insights into the correlation between the mechanical properties and crystallite size of silk fibres. It is reported that the critical dimensions of crystallite for optimal mechanical properties of
B. mori are 2-4 nm along interchain direction and 1-2 nm along the strand axis [109]. Spider silk that has β-sheet crystallites about 6.5 nm and 3 nm along the two directions, respectively, have the optimal mechanical properties [110]. So far, experimental work to correlate the size of β-sheet crystallites with the mechanical properties of A. pernyi silk fibres has not been reported.

Apart from crystallite size, orientation of crystallites, which can be characterised by polarized optical microscopy (birefringence) [111] and 2D XRD (Hermans orientation function) [22], is another important parameter that affects the mechanical properties of a silk fibre. High strength of a silk fibre can be obtained by increasing the degree of orientation of the crystalline structure [22]. Spider silk has well oriented crystallites with an orientation function of 0.98, which contributes to its outstanding mechanical properties [106]. In contrast, the crystallites of natural A. pernyi silk have an orientation function of 0.35, which can be significantly improved to 0.96 by reeling speed of 20 mm/s [22]. Thermal annealing can affect the orientation of crystallites the fibre at a speed of 20 mm/s. As shown in Figure 1.12, the birefringence of A. pernyi fibres is lower than that of B. mori fibres in the temperature range from 25 to 260 °C. In addition, an increase in temperature leads to a more significant reduction of the birefringence of A. pernyi silk fibres [112]. These indicate that the molecular orientation of A. pernyi silk is remarkably lower than that of B. mori silk, but molecular rearrangements of this wild silk is more likely to be induced by thermal treatment. However, the changes in molecular rearrangement and their effects on the mechanical properties of silk fibres upon thermal treatment have not been investigated.
1.3.5 Factors influencing mechanical properties

According to the breaking mechanism, the mechanical properties of a single fibre can be tuned by manipulating the amount and ratio of different motifs. Silk fibres have been bestowed with different tensile strength by changing reeling speed [5, 91], solvents for degumming [13], moisture/humidity [113], and temperature [102]. All these affect the amount and ratio of the motifs.

(1) Reeling speed

By increasing reeling speeds, random coils in *A. pernyi* silk fibres [84] could be converted into β-sheets upon stretching, which increases molecular orientation and improve tensile strength but decrease tenacity of the fibres [114]. At the same reeling
speed, *A. pernyi* silk with a higher content of random coil has lower stress but higher strain than that of *B. mori* silk [84].

(2) Silk degumming

Degumming always has an adverse effect on the breaking stress of silk fibres. Degumming degrades silk proteins and reduces the crystallinity of silk fibres [115]. Hence, $\alpha$-helix and random coil are the main molecular conformations in degummed silk fibres [45, 50]. Degumming has a larger negative effect on the tensile strength of a silk fibre, but little influence on its elongation to break. Although the strength can be recovered partially by converting some $\alpha$-helix and random coil into $\beta$-sheet structure via suitable treatments, such as water vapour annealing, immersing in ethanol, acetone or isopropanol [92, 116], its elongation further decreases remarkably due to great reduction of amorphous content. Different degumming methods can reduce the mechanical properties to different extents [13], depending on the degree of conversion among the different conformations.

(3) Humidity/moisture

Besides silk degumming, the mechanical property of silk has also been found to be negatively influenced by humidity, with a reduction of modulus and glass transition [117]. That is because water reduces the number and strength of hydrogen bonds between $\beta$-chains, and thus greatly weakens the strength of silk fibres [118]. The decrease of intermolecular hydrogen bonds may reduce the strain-weakening zone (Figure 1.11)[9] and therefore cause breaking of $\beta$-crystallites in silk fibres.

(4) Temperature
Two temperatures, glass transition and melting temperature, are responsible for the mechanical properties of silk fibres. *B. mori* silk has a melting point in the range of 308 - 417 °C [119, 120]; whilst *Nephila* dragline silk show excellent heat resistance, falling mechanically only at 371 °C [120]. Shao et al. reported that when cooled to -60 °C, the strength of spider silk fibres was increased and the elongation to fracture could be doubled [120], probably due to the relaxation of hydrocarbon-hydrocarbon interactions [121]. The effects of temperature on the mechanical properties of *A. pernyi* silk fibre have not been investigated. How the temperature affect the structure of the fibres and hence the properties is worthy of investigation.

1.4 Thermal properties of *Antheraea pernyi* silk cocoon and fibres

Thermoregulation by silk fibres and cocoons are important to the survival of silkworms in particular those of wild spices. The excellent thermoregulation properties of silk fibres also make silk fabrics a popular choice for consumers.

Knowing the thermal conductivity/diffusivity of fibres, such as silk, can be a key to obtaining the optimum performance for a particular application.

1.4.1 Theoretical basis and definitions

The basic ways for heat transfer are convection, radiation and conduction. Convection is a main way of heat transfer in fluid and gas phases. It can be ignored between fibres and the interstitial fluid as the convection motions could be retained by them. Radiative heat transfer is important in high temperature applications, such as light in rectilinear propagation. Conduction is the main way for heat transfer in solid material [122]. As the inherent parameters, the thermal conductivity $k$ (under steady-state conditions) and
thermal diffusivity $\alpha$ (under transient conditions) denote the capacity of a material to conduct heat [123]. The relationship between them is as follows [124].

$$\alpha = k / \rho C_p$$  \hspace{1cm} (Equation 1-1)

where $\alpha$ is thermal diffusivity; $k$ is thermal conductivity; $C_p$ is the specific heat and $\rho$ is the density of a material.

Thermal conductivity is the quantity of heat that passes in unit time through one square metre and one metre thickness when its opposite side differ in temperature by one kelvin. The equation that governs the heat flow is known as Fourier’s law, shown as follows,

$$k = -\frac{q}{A} \frac{dx}{dt}$$  \hspace{1cm} (Equation 1-2)

where $q$ is heat flow; $A$ is the cross sectional area normal to the x direction and $T$ is temperature.

Thermal diffusivity is valued by the variation of temperature distribution versus time. It is appropriate for many materials to reach thermal equilibrium within a short time. These materials include single-layer graphene [125], organic aerogels [126], carbon fibre [127], B. mori silk fibre [128], spider silk fibre [8], and slate [124]. After the temperature evolution $T \sim t$ of a material is obtained, the thermal diffusivity by phonon transport can be obtained in a temperature gradient [129].

To characterise $\alpha$ and $k$, a number of techniques have been developed over the past several decades, such as, hot plate [122] and laser flash for bulk materials, electrothermal technique (i.e. 3ω method [130], closed circuit [128] and wheatstone bridge circuit [131]) and technique assistance (i.e. Atomic Force Microscopy (AFM) [132] and Raman spectroscopy [125]) for micro/nano fibres.
1.4.2 Thermal insulation of silk cocoons

Thermal conductivity of silk cocoons has been measured via the typical steady state method in the thickness direction [133]. The *A. pernyi* cocoon exhibits a slightly higher level of thermal transfer than that of *B. mori*, which is probably attributable to the calcium oxalate deposits on the *A. pernyi* cocoon [65]. Figure 1.13 shows the thermal conductivities of several kinds of cocoons and calcium oxalate crystals.

![Figure 1.13](image.png)

**Figure 1.13** Thermal conductivity of different silkworm cocoon walls [65]

1.4.3 Thermal behaviour of one dimensional fibres

Due to its low dimensions, it remains a challenge to characterise the thermal conduction along a single fibre. Thermal conductivity is one of the most important properties to evaluate the heat transfer characteristics of a fine fibre, like carbon fibre [127, 134], metallic and non-metallic fibres, etc. The thermal transportation of single micro/nano wires, especially those with very low thermal conduction such as natural
fibres like silk, wool and cotton, is very challenging. Though some researchers have reported the thermal conductivities of a single fibre like *B. mori* (0.54-6.53W/mK) [128], spider silk (150-300W/mK) [8], polyethylene nanofiber (~104W/mK) [132] and carbon fibre (~240W/mK) [127], the results are not reliable due to the large fluctuation (one order of magnitude) of the results. In addition, a comparison between the thermal properties of different fibres has not been possible, since different techniques were adopted for the measurements. To date the effect of crystallite size on thermal transfer in a single *A. pernyi* silk fibre has not been investigated. To evaluate the thermal properties of a single *A. pernyi* fibre against those of other natural fibres, a same technique has to be adopted.

1.4.4 Phonon heat transport in a single fibre

Generally, electrically insulating materials, like protein fibres, are phonon-based heat conductors. When heat is applied at one end of a single fibre, the heat flux run along the chain of polypeptide with gradually decreasing temperature. When multi-chains form a nanofiber or a bulk material, chain alignment becomes critical for heat transfer. An ultra-drawn polyethylene nanofiber has demonstrated a high thermal conductivity of 104 W/m·K [132], mainly due to the enhanced phonon transport in mechanically aligned polymer chains. Stretched silkworm fibres have higher thermal conductivity due to the more ordered alignment of β crystals and random coils, which decrease phonon scattering [128]. While, spider silk fibres, stretched or not, have surprising thermal conductivity up to 416 W/m·K [8]. Although the crystallinity of spider silk is lower than that of silkworm silk fibres, the arrangement of molecular chain in non-crystalline region of spider silk fibres is more regular, leading to less scattering of phonon [31]. That means crystallinity and
orientation have coupled effects in thermal transfer. **Figure 1.14** shows the structure of various fibres.

![Figure 1.14 Schematic structure of various fibres [135]](image)

As the crystallinity and orientation are also related to mechanical properties, thermal conductivity/diffusivity can be correlated with mechanical properties and micro-/nano-structures, to analyse the thermophysical properties of protein fibres. According to a widely used kinetic model \( k \sim \rho C_p v l = C_p l \sqrt{E\rho} \), the thermal conductivity/diffusivity of silk can be modulated by tuning its Young’s modulus \( E \).

### 1.4.5 Thermal conductivity of a single crystal

Phonon is quanta of lattice vibrations, which plays an important role in thermal conduction in solid state. As the main contributors to phonon conduction, crystallites are critical to thermal conduction of natural fibres. The thermal conductivity of single cellulose nanocrystals is reported as \(~0.72-5.7\) W/m·K [136]. Little study has been reported on the thermal conduction of silk nanocrystals. The recent study investigated by Zhang *et al.* demonstrates that with the increasing number of \( \beta \)-strands, the thermal conductivity of a single \( \beta \)-sheet from spider silk increases from \( 1.85 \) to \( 4.30\) W/m·K.
It is widely accepted that the *A. pernyi* crystallite consists of several anti-parallel β-sheet connected by van der Waals force, however, the thermal conductivity of single crystals and how heat transfers in these crystals has not been investigated.

According to Figure 1.14, the crystalline β-pleated sheets made up of several antiparallel peptide chains should contribute much for efficient heat conduction. Structurally, the *A. pernyi* β-sheet is equivalent to a network of alanine residues connected by intra-strand covalent bonds and inter-strand hydrogen bonds. Lin reported that increasing the covalent bonds and inter-hydrogen bonds [138], the thermal conduction of nylon [139] and spider silk [137] was improved. In addition, the crystallite size is also influenced by bond length. Bond length is proportional to bond energy [132, 140], which may be correlated with the thermal conduction.

Any structural modification performed to enhance thermomechanical properties of a material will inherently affect its heat transfer properties. As discussed in section 1.3.4, there are critical dimensions of crystallite for optimal mechanical properties. It is still unknown how crystallite size affects the thermal conduction of silk fibres.

1.4.6 Crystalline structure versus thermal property

(1) Mean free paths of phonons (*l*<sub>ph</sub>)

According to a widely used kinetic model, \( k \sim \rho C_p \vartheta l_{ph} = C_p l_{ph} \sqrt{E \rho} \), improving Young’s modulus \( E \) may enhance thermal conductivity (\( \rho \): density; \( C_p \): specific heat; \( \vartheta \): sound speed; \( l \): phonon mean free path) [8]. The thermal conductivity of a material is also related to the mean free path of phonons \( l_{ph} \). At low temperatures (below a temperature near the dielectric maxima), the phonon mean free path is limited by boundary scattering due to the combined effects of lattice defects and the crystallite size and hence, influence the thermal conductivity of materials [140].
(2) Crystallite size

To highlight the effect of crystallite size, Figure 1.15 shows $k$ as a function of the number of β-strands ($n$), β-sheet layers ($l$), the length of individual poly-A β-strands ($L$) of silk protein [137, 141] and the average grain size ($D_{\text{avg}}$) of nanocrystalline silicon [142]. At room temperature, increasing the number of β-strands and the length of poly-A β-strands enhances the thermal conductivity of silk proteins until equilibriums are reached, whereas the number of β-sheets influences less on thermal conductivity. The recent study focused on crystalline silicon indicates that, thermal conductivity generally increases along with the increase of grain size (Figure 1.15 (d)) [142]. Considering the temperature effect, bigger grain size does not necessarily lead to better thermal conductivity. However, how the crystallite size of A. pernyi silk fibres affect their thermal transfer has not been understood.
Figure 1.15 Thermal conductivity vs the number of $\beta$-strands (a), the number of $\beta$-sheets (b) [141] and the length of individual poly-A $\beta$-strands (c) [137] of silk protein at room temperature, as well as the average grain size of nanocrystalline silicon (d) [142]

(3) Anisotropic bond chemistry

The anti-parallel nano-crystallite in silk is actually a kind of orthorhombic crystal [143]. It includes the three dimensions along $a$, $b$ and $c$ axis. $c$-axis is parallel to the fibre axis, $a$ is the direction along the stacking of $\beta$-sheets and $b$ is the direction in a $\beta$-sheet perpendicular to the strand axis (as shown in Figure 1.16). The bond chemistry is anisotropic for the three directions: the stiff covalent bonding along the chain backbone, the hydrogen bonding along the inter-chain direction, and the weak van der Waals bonding in the lateral directions.
From a fundamental perspective, the strong anisotropic bond chemistry gives rise to an interesting size effect on thermal conductivity. To date, the basic picture depicted by this area of research is that shrinking the size of crystalline materials such as nanowires and thin films results in lower thermal conductivity because of increased phonon scattering at boundaries [145]. However, some nanostructures, like carbon nanotubes [146], exhibit starkly contrasting behaviour. The reduction in dimensionality of carbon nanotubes limits phonon scattering that leads to longer phonon mean free paths and higher thermal conductivity. This is also the case with polyethylene [147], which has a lower thermal conductivity with more chains added to form a lattice plane, due to the anharmonic scattering induced by neighbouring chains. It is worth of investigating the variations of a single crystal size on the thermal conduction of silks. A single chain is expected to have a higher thermal conductivity, a transition from 1D to 3D can give a clue about the effect of crystal size and the chemistry bonds (i.e. hydrogen bond, covalent bond and van der Waals) on thermal conduction.
1.4.7 Other factors influencing the thermal property of silk fibres

(1) Temperature

Temperature has a great influence on the thermal conductivities of different materials. Different materials show different temperature-dependent thermal conductivities [148-150]. It is important to understand the effects of temperature on thermal conductivity of materials for various applications.

It is noted that for electrically insulating materials, the thermal conductivity of the nanowire behaves similarly to their bulk counterparts [151]. However, for thermal transport of materials based on phonons and electrons, one dimensional wires and bulk such as silver nanowires and bulk silver have different temperature-dependent thermal conductivities especially at low temperatures (Figure 1.17) [150]. At a lower temperature, the phonon-electron scattering is lower, so the thermal conductivity of bulk silver is higher at a lower temperature; however, the structural scatterings still exist for silver nanowires and dominate the electron transport. Therefore, the thermal conductivity of a silver nanowire increases with temperature.
Figure 1.17 Thermal conductivities of silver nanowires and bulk silver [150]

(2) Specific heat capacity

The specific heat capacity is a measurable physical property. It is the amount of heat needed to increase the temperature of one gram of a substance by one degree. At temperature below \( T_p \), the specific heat capacity of noncrystalline silks was found to be linearly correlated to temperature very well (correlation coefficient is 0.992).

\[
C_p(T)_{\text{solid}} = 0.130 + 3.70 \times 10^{-3} \ T \ [J/(g \times K)]
\]  

(Equation 1-3)

As shown in Figure 1.18, the increment of \( C_p \) was also found to be linear fitted with the \( \beta \)-sheet content.
Figure 1.18 The $\Delta C_p$ increment at $T_g$ vs beta-sheet content determined from FTIR

(3) Porosity/density

A porous structure effectively decreases the net-section area which provides the transport path for phonons [152]. The presence of pores reduce thermal conductivity. For example, the thermal conductivity of silicon with a porosity of 50% is 1 W/mK, which decreases to 0.8 W/mK at a higher porosity of 64% [153]. Along with porosity, the pore shape, pore size and size distribution also affect the thermal conductivity [154]. For example, spherical pores perpendicular to the heat flux is more effective for promoting thermal transfer than prolate pores of equivalent volume [155]. The thermal conductivity/diffusivity of some materials can also be enhanced by reducing its density (Figure 1.19 (b)). This can be explained by the increased radiative and/or convection heat transfer.
Figure 1.19 Porosity dependence of the thermal conductivity (a) [156] and the thermal conductivity of a mineral fibre insulation versus packing density, measured using a guarded hot plate (b) http://www.ngb.netzsch.com/

*A. pernyi* silk fibre is characteristic of porosity in its transverse section. It can be predicted from Figure 1.19 (a) that, the pores inside the *A. pernyi* silk fibres have negative effects on thermal conductivity, and this thermal conductivity could be tuned by the porosity.

(4) Moisture

In general, increasing moisture content can increase the thermal conductivity of materials. For example, it has been reported that the thermal diffusivity of spider silk decrease by more than half (6.4→2.7 × 10⁻⁵ m²/s) after moisture adsorption, with a length decrease of ~24% and a ~11% (4.734→4.237 μm) increase in diameter [8]. Due to the increased hydrogen from wetting, the moisture would increase specific heat and density, and therefore, there will be a smaller decrease for thermal conductivity, $k$. 
1.5 The interactions across protein-protein interfaces

As a natural composite structure, the excellent mechanical properties of silk fibres are not only affected by the properties of fibroin and sericin, but also dependent on the interfacial adhesion between these two components.

1.5.1 Surface topography and surface energy

The interfacial adhesion between fibroin and sericin is partly due to the surface topography of fibroin fibres. As discussed in section 1.2.9, distinct longitudinal ridges and striations are present along the fibre axis of wild silk fibroin fibres, whilst *B. mori* fibroin fibres have very smooth surface. The rough fibroin fibres of wild silk may facilitate the adhesion of sericin on their surface. Compared to the fibroin fibres of *B. mori*, the higher value and wider variation of dispersive surface energy of *A. pernyi* fibroin fibres are also indicative of more energetic sites for better interactions with the adsorbate [95]. This may contribute to the difficulties in degumming *A. pernyi* fibres.

The interactions between fibroin and sericin, which may help to explain the hard degumming of this wild silk, have not been understood. Due to their excellent biocompatibility, much efforts have been devotes to the interactions of these proteins with other macromolecules such as cellulose [157, 158], chitosan [159], and nylon 66 [160] for fabrication of fibroin- or sericin-based composite materials that are ductile, mechanically strong and biocompatible.

1.5.2 Techniques for charactering the interactions between fibroin/sericin and other substances

(1) Structural characterizations
Pulsed-field gradient nuclear magnetic resonance (PFG-NMR) is a well-established method for the determination of translational diffusion coefficients. The optimum concentration ratios of the both sides (carrier and acceptor) for interactions can be obtained in order to observe maximum changes in the carrier diffusion coefficient upon binding. In addition, the commonly exploited parameters like chemical shift, Nuclear Overhauser Effect (NOE) and diffusion measurements can be applied to detect the binding site, conformation changes, and even binding to a target acceptor [161]. It can determine quantitatively the conformations, which can be also achieved by Raman, FTIR, XRD and CD, etc, because they have the corresponding attributions of amides to detect. These techniques have been applied to investigate the interactions between fibroin protein and copper (II) [162], chitin [163] and poly(ethylene oxide) (PEO) [164] etc.

(2) Fluorescent technique

Fluorescence measurement can be used to determine the changes in hydrophobicity and conformational transitions during the interaction process. The fluorescence intensity of B. mori fibroin protein can be recorded with excitation wavelengths of 490 nm and 570 nm [165]. The excitation wavelengths of A. pernyi fibroin protein are set to be 420 nm and 340 nm with Thioflavine T (ThT) and pyrene as the fluorescence probes, respectively [166]. Any interaction between fibroin and other substances will bring about a pronounced quenching of the fibroin fluorescence.

(3) Scanning Probe Microscopy (SPM) technique

SPM is the microscopy techniques including scanning electron microscope (SEM), atomic force microscope (AFM) and optical microscope, etc. This technique has been prevalently applied to detect the interactions between materials macroscopically and
microscopically. The macroscopic observation can be achieved by changes in surface morphology, whereas the microscopic variation can be detected by phase interface (AFM), ion penetration (EDX or XPS) or orientation (optical microscope), which has been used to study the interactions between *B. mori* fibroin and poly(ethylene glycol) (PEG) [167].

(4) Surface plasma resonance (SPR) and Quartz crystal microbalance (QCM)

SPR and QCM are two powerful label-free detection techniques for detecting interactions between molecules or molecules with surfaces. SPR is a static measurement for the conformational change of molecules bound to an adhesive surface. QCM is basically a mass loading sensor for dynamic measurement. The dissipation of mechanical energy can be obtained due to the internal friction of the adsorbed layer. *B. mori* silk fibroin protein has been monitored by SPR and QCM for multilayers buildup [168], adhesion onto titanium substrate [169], and the interaction with plasma proteins [170]. To date, little information in this aspect is available for the interactions between fibroin and sericin.

(5) Molecular dynamic simulations (MD simulations)

Studies reveal that biomolecules undergo structural change upon interacting with other molecules or substrates. These structural modifications occur mainly at the molecular level, involved in amorphous, crystalline and segments from N-terminal. MD simulations have been widely utilised to investigate the interactions of biomolecules such as proteins and peptides with other molecules/ions [171] and various types of nanoscale materials, such as graphene [172].
They are also many other techniques to investigate protein-protein interactions, such as dynamic light scattering [173] and isothermal titration calorimetry [174], etc. Each of these techniques has its own strengths and weaknesses, especially with regard to the sensitivity and specificity of the method.

1.6 Summary

This literature review summarised the silk cocoon variety, structure and properties for protecting *A. pernyi* silkworm pupae from extremes of climate conditions and predators, as well as the parameters that affect physical (mechanical and thermal) properties of silk fibres. This chapter draws the main research question, which is ‘what are the differences in the structures and properties of the key cocoon components, *i.e.* peduncle, outer floss and cocoon fibres that contribute to their special functionalities’. Understanding the fibre design and its ability to provide protection can help us design future functional fibrous materials and also provide guidance to design of fibres with desirable properties for different applications. The work conducted in this research is organised into four chapters, as shown below.

In chapter 2, silk fibres from different parts of *A. pernyi* cocoon, *i.e.* peduncle, outer floss, outmost fibre and innermost fibre, are firstly studied to correlate the fibre structure with their corresponding mechanical properties.

In chapter 3, the influences of temperature on the nanocrystalline structure of silk fibres and consequently on the mechanical properties of the fibres are studied, through a comparison of the structures between different parts of *A. pernyi* and *B. mori* silkworm silk. The refinement of nanostructure by annealing treatment is helpful to develop silk fibres with tuneable mechanical properties.
In chapter 4, the thermal properties of raw silk layers from *A. pernyi* and *B. mori* are studied and the constituents including fibroin fibre, sericin and calcium oxalate are analysed to illustrate their contributions to the overall thermal conductivity of a cocoon. Thermal conduction of single fibres in transverse and longitudinal directions is also investigated to illustrate the relationship between crystallite and thermal conductivity/diffusivity. These experimental procedures can be applied to analyse the nano-structure and thermal properties of many fibres, such as cotton, wool, and silk. It may further guide the design of synthetic silk materials with tuneable thermal properties.

In chapter 5, the different types (*i.e.* specific and non-specific) of adhesion between fibroin and sericin of *A. pernyi* and *B. mori* are investigated. The findings account for the reason of hard degumming of *A. pernyi* fibres and would help develop suitable silk processing, such as silk degumming methods, and inspire new biomimetic composite materials.

The key results of this project and outlook for future work are summarised in chapter 6.
CHAPTER 2 Microstructure and mechanical properties of silk fibres from different parts of the *A. pernyi* cocoon

2.1 Introduction

As explained in literature review, as a unique habitat of *A. pernyi* silkworm, the morphology and structure of *A. pernyi* cocoon decide its protective functions, such as exceptional mechanical, thermal regulation [65, 68, 175] and UV screening properties [71]. The out-of-plane compressive modulus and hardness of the *A. pernyi* silk cocoon wall are superior to those of the *B. mori* silk cocoon wall [69]. The in-plane tensile strength of *A. pernyi* cocoon is much higher than the out of plane strength [103, 104]. *A. pernyi* cocoon shows a significant buffer against environmental temperature changes [176] and it is also an effective shield to pupae from UV radiation [71]. Studies conducted on the *A. pernyi* cocoon in the past few years have been limited to the cocoon shell and fibres from it [69, 71, 103, 104, 177]. The structure and properties of fibres from the other parts of the cocoon have not been investigated. In addition, despite of molecular modelling and simulation, there has been no reported experimental research to correlate the size of β-sheet crystallites to the mechanical properties of silk fibroin fibres.

It has been mentioned in chapter 1 that a light weight and compact *A. pernyi* silkworm cocoon includes peduncle, outer floss and the cocoon shell. The cocoon shell consists of intersecting multiple layers of fibres. The innermost layer is compact and smooth. The external layer is termed as the outermost layer which is flossy. The outer floss refers to the loose layer of fibres surrounding the cocoon shell (See Figure 2.1). Its
thickness is $0.055 \pm 0.009$ mm. The peduncle ($0.412 \pm 0.014$ mm thick), which serves to hang the cocoon on twigs, is absent in domestic cocoons. It is a unique feature of some wild silkworm cocoons. The peduncle and the outer floss are generally discarded in industrial silk processing, probably due to their small mass (~ 14 wt% of the cocoon) and the difficulties to spin them.

![Figure 2.1 Photos of the A. pernyi silk cocoon (a) and its anatomical diagram (b)](image)

A thorough understanding of the microstructures and physical properties of fibres from these three main parts of the A. pernyi cocoon is essential for revealing the protective mechanisms of the cocoon. The insight gained may help guide the design of materials and structures with desirable properties for protective applications. Meanwhile, it can also promote the use of silk fibres from peduncle and outer floss.

This chapter aims to understand the differences of the structure and physical properties of silk fibres from the three parts of the A. pernyi cocoon at micrometer and nanometer scales. Their structural morphologies are observed by confocal microscopy and scanning electron microscopy (SEM). Fibre diameters are obtained from Single fibre analyser (SIFAN). The physical performance of different silk fibres is examined by
Chapter 2 Microstructure and physical properties of silk fibres from *A. pernyi* cocoon
tension test, temperature-dependence DMA, differential scanning calorimetry (DSC), FibreStress tester and nano-indentation test. Fibres from *B. mori* cocoon are also used as a comparison where appropriate.

2.2 Experimental

2.2.1 Silk fibre sampling

*A. pernyi* silkworm cocoons were obtained from Liaoning province, China. Silk fibres are gently drawn from the peduncle, the outer floss and the cocoon shell (including outermost layer and inner layer, respectively) [91]. The linear density of fibres from each part is calculated based on Tex (the weight in grams of fibres with 1000 m length) by weighing fibres with known lengths. Silk fibres are also degummed for comparisons of physical properties. Degumming is performed using 0.5% sodium carbonate solutions at 98 °C for 1 h. The solution from degumming is dialysed and freeze dried to obtain sericin.

2.2.2 Air permeability of *A. pernyi* silk cocoon

Cocoon shell is equally divided into three layers (0.156 mm for each layer) and cut into circular disks with 30 mm in diameter. The air permeability is examined by the FX 3300 Air Permeability Tester III (TEXTEST) according to the standard (SIST EN ISO 9237 - 1999).

2.2.3 Surface and cross-sections of silk fibres from different parts

The morphology of silk fibres is examined by confocal microscopy. Silk fibres conjugated with a fluorescence dye (Tetramethylrhodamine-5 (and 6)-Isothiocyanate (TRITC)) are observed under a confocal laser scanning microscope (Leica SP2 AOBS
Laser). A field emission scanning electron microscope (FEG-SEM) (Zeiss Supra 55VP), at an acceleration voltage of 5 kV, is also used to characterise the surface and cross sections of fibres. To achieve this, silk fibres are embedded in epoxy resin and cut with a LBK Ultramicrotome to get thin slice (~ 500 nm) of fibres. The sericin contents and volume fraction of pores are calculated via differential and integral calculus method. Their mean values are taken from at least 10 cross sections for fibres from each part.

2.2.4 Single fibre analyser (SIFAN)

The diameter of silk fibres is obtained from SIFAN with a gauge length of 25mm and head speed of 15 mm/min. Results from at least 100 fibres are averaged for fibres from each part. Finally fibres with diameters of 15 ~ 17 μm are chosen to compare the degumming effect on the abrasion resistance of *A. pernyi* silk fibres.

2.2.5 Abrasion fatigue measurement

Single fibre abrasion and bending fatigue is tested on a FibreStress tester (Textechno). 100 fibres for each type from peduncle, outer floss and cocoon shell are tested under standard conditions of 20±2°C and a constant humidity of 60±2% RH, respectively. Fibres are traversed over a stainless steel wire in a 90° arc under 0.8 g tension with an amplitude of 10 mm and a frequency of 2 Hz.

2.2.6 Tensile tests of silk fibres

All samples are preconditioned for at least 24 hours at ambient temperature of 20±2 °C and a constant relative humidity of 65±2 % before measurements. Mechanical testing is carried out on an Instron 30 kN with a gauge length of 25 mm and cross head speed of 18 mm/min. At least 100 fibres from each cocoon part are tested. The load –
displacement curves are converted into stress-strain curves, from which the elastic modulus \( E \) is calculated.

2.2.7 Temperature-dependent dynamic mechanical properties

Dynamic mechanical properties of fibres including storage modulus \( E' \), loss modulus \( E'' \) and the loss factor \( \tan \delta \) (the ratio of \( E'' \) and \( E' \)) are examined on a dynamic mechanical analyser (DMA) (Q800, TA Instrument Netzsch, Germany). All samples are scanned at a ramping rate of 3 °C/min in the temperature range of 50 - 350 °C at 1 Hz. For fibres from each part, 20 measurements are performed under isochronal conditions and the results are averaged.

2.2.8 Measurement of fibre hardness

Indentation experiments are performed perpendicularly to the plane direction of fibres, using an IBIS nanoindentation system (UMIS II, Australia). A force of 5 mN is applied by a Berkovich-type indenter (a three sided pyramidal diamond tip) to determine the hardness and the elastic modulus. At least 100 loading-unloading tests are performed on silk fibres from each part, and the average values are taken. The penetration depths for all samples are smaller than 1 μm, which are far below one-tenth of the thickness of the samples to avoid the supporting effects of substrate. Hardness and elastic modulus are obtained by the built-in analysis software according to the Oliver-Pharr (O-P) method [178]. The area function for Berkovich indenter is determined through preliminary indentation tests made on fused quartz.
2.3 Results and discussion

2.3.1 Surface morphology

As shown in Figure 2.2, silk fibres from peduncle, outer floss and cocoon shell (outermost layer and inner layer) of the *A. pernyi* cocoon have different morphologies. Peduncle fibres are closely packed and aligned along the fibre longitudinal direction. Outer floss and cocoon fibres are randomly distributed like a non-woven fabric. From the outermost to the inner layers, the fibres become more densely packed (Figure 2.2 (c, d)). The variation in fibre density offers silk cocoon gradient air permeability through its thickness direction. Outermost layer has the best air permeability of about 12.95 cm$^3$/cm$^2$/s, whereas those of the middle and inner layers with the same thickness are about 8.50 and 2.81 cm$^3$/cm$^2$/s, respectively. The air permeability decreases from the outermost to the inner layers due to the increasing compactness of silk fibres. The increasing fibre density may be also related to the increasing protection level against physical attack towards the inner surface of cocoon.

![Figure 2.2](image)

**Figure 2.2** Confocal images of silk fibres labelled with a fluorescence dye tetramethylrhodamine-5 (and 6)-isothiocyanate (TRITC)
Chapter 2 Microstructure and physical properties of silk fibres from *A. pernyi* cocoon

**Figure 2.3** SEM images of natural *A. pernyi* silk fibres

**Table 2.1** The geometrical parameters and physical properties of silk fibres from different parts of the *A. pernyi* cocoon

<table>
<thead>
<tr>
<th>Silk fibres</th>
<th>Diameter / μm</th>
<th>Linear density / tex</th>
<th>Sericin thickness / nm</th>
<th>Volume fraction of sericin / %</th>
<th>Porosity / %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peduncle</td>
<td>15.53 ± 3.54</td>
<td>0.32 ± 0.01</td>
<td>150 - 350</td>
<td>7.27 ± 1.7</td>
<td>4.45 ± 1.2</td>
</tr>
<tr>
<td>Outer floss</td>
<td>20.16 ± 2.96</td>
<td>0.44 ± 0.04</td>
<td>500 - 650</td>
<td>11.98 ± 2.5</td>
<td>4.11 ± 0.8</td>
</tr>
<tr>
<td>Outermost fibre</td>
<td>21.24 ± 2.94</td>
<td>0.49 ± 0.00</td>
<td>250 - 820</td>
<td>11.90 ± 2.1</td>
<td>0.41 ± 0.1</td>
</tr>
<tr>
<td>Innermost fibre</td>
<td>16.28 ± 2.16</td>
<td>0.35 ± 0.01</td>
<td>150 - 355</td>
<td>6.85 ± 2.3</td>
<td>1.40 ± 0.5</td>
</tr>
</tbody>
</table>
The SEM images in Figure 2.3 show the morphology, cross section of silk fibres from different parts at various scales. The corresponding geometrical parameters and physical properties of silk fibres are given in Table 2.1. Similarly, silk fibroin fibres from different parts are held together in pairs by the sericin gum and impurities. However, the cross sections of the fibres have big differences. According to Figure 2.3, the cross-sections of peduncle and cocoon fibres are typically triangular that is in good agreement with literatures [179, 180]. The cross sections of outer floss fibres are not uniform in shape. Both triangular and elliptical shapes were observed. In contrast to B. mori, A. pernyi silk fibres are characteristically porous, as seen from the cross sections. As stated in Chapter 1, these pores are produced by vesicles in its posterior region [15] and elongated during spinning through the spinneret and persist in the silk fibres [181]. This porous structure should contribute to the excellent thermal insulation property of cocoons in the wild [152, 153]. The pore shape, pore size, as well as porosity are different for fibres from the different parts. The size of pores decreases progressively from cocoon fibres to outer floss and peduncle fibres. Fibres from outermost layer and innermost layer of cocoon have larger pore sizes (around 37,000 nm\(^2\) and 22,000 nm\(^2\), respectively) but lower porosity (0.41% and 1.40%, respectively). Peduncle fibres have small pores (around 12,000 nm\(^2\)) and the highest porosity of 4.45%. These micro-voids lower fibre density and weaken inter-fibrillar interactions [182]. Outer floss and outermost fibres have a relatively large volume fraction of sericin content. This could also be reflected from the decrease of fibre diameter before and after degumming (See Figure 2.4). The difference in pore size, porosity and sericin content will undoubtedly contribute to the differences in the mechanical and thermal properties of the fibres.
2.3.2 Tensile strength of silk fibres

*Table 2.2* and *Figure 2.5* shows the mechanical properties of silk fibres from the different parts of *A. pernyi*. It is assumed that two adjacent monofilaments contribute equally to the tensile strength of a single fibre [183]. Herein the tenacity of one monofilament is obtained by averaging the value of the twin fibres. The data in this paper is all based on one monofilament.
Table 2.2 Tensile properties of silk fibres from different parts of *A. pernyi* cocoon

<table>
<thead>
<tr>
<th>Fibres</th>
<th>Peduncle</th>
<th>Outer floss</th>
<th>Outermost fibres</th>
<th>Innermost fibres</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tensile stress (MPa)</td>
<td>455 ± 52</td>
<td>596 ± 105</td>
<td>773 ± 166</td>
<td>746 ± 102</td>
</tr>
<tr>
<td>Elongation (%)</td>
<td>31.11 ± 7.44</td>
<td>44.74 ± 8.47</td>
<td>51.08 ± 11.44</td>
<td>36.84 ± 6.20</td>
</tr>
<tr>
<td>Modulus, GPa</td>
<td>5.67 ± 0.84</td>
<td>6.15 ± 1.23</td>
<td>4.7 ± 1.85</td>
<td>5.23 ± 0.62</td>
</tr>
</tbody>
</table>

Figure 2.5 Representative tensile curves of different raw silk fibres from *A. pernyi* cocoon. (1) Peduncle; (2) Outer floss; (3) Outermost fibre; (4) Innermost fibre. The insert figure shows the stress-strain curves of degummed silk fibres of *A. pernyi*
Chapter 2 Microstructure and physical properties of silk fibres from *A. pernyi* cocoon

Although the tenacity of the fibres from outer floss and cocoon is similar to that of fibres from peduncle, their breaking strains are much higher. The resulting high toughness can help fibres from the outermost layer absorb the largest breaking energy, which helps to protect the pupa inside. Peduncle fibres, in spite of their low strength and elongation, have a Young’s modulus as high as that of innermost fibres. This indicates that peduncle fibres are not easily deformed, with good rigidity under a small load. Fibres from innermost layer, have a relatively high strength but low elongation, probably due to their low porosity and low sericin content.

The outer floss and fibres from the outermost layer have similar sericin content and linear density, thus the difference in strength and breaking length should be related to their internal structures such as porosity. The large porosity of outer floss fibres can weaken the interaction between silk microfibrils and scatter the stress area, leading to low strength and elongation [184]. The high porosity of peduncle fibres may also account for their inferior mechanical performance.

The inserted figure of degummed silk fibres (Figure 2.5) shows that degumming results in significantly weak strength (from 450 ~ 800 MPa to 180 ~ 350 MPa), whilst elongation is less affected. Compared to peduncle and outer floss fibres, degummed silk fibres from cocoon (including outermost and innermost fibres) show an obvious reduction in strength (from ~750 to ~300 MPa), indicating deterioration of fibroin fibres caused by degumming.

2.3.3 Bending abrasion of silk fibres

To understand the mechanism of silk fibre failure, bending abrasion tests are performed using a FiberStress tester. Silk fibres with diameter of about 15 um are selected to differentiate the five kinds of fibres and the results are shown in Figure 2.6.
As shown in Figure 2.6, outer floss and outermost fibres have a relatively high abrasion resistance, followed by innermost fibre and peduncle. The raw *B. mori* silk fibre can withstand only 1% of the number of cycles required to break the outer floss/outermost fibres. The difference could be due to the differences in surface and structure of fibres. The cubic calcium crystals on outermost/outer floss fibres will make it harder to break. The high sericin content in amorphous structure makes *B. mori* fibres easier to break.

It is apparent that there was a significant increase in fibre failure rate after silk degumming, especially for the outermost fibres. This is probably due to the removal of calcium oxalate crystals, as the fraction of which reduces during silk degumming process. Outer floss still maintains the best abrasion resistance after sericin removal. This abrasion behaviour indicates that fibril cohesion is strong in the fibre system, and
it causes the shear of the fibre itself. In lateral abrasion cycles, frictional forces are able to displace fibrils from their normal position, and these fibrils ruptures through bending and flexing. The superior abrasion resistance of raw silk fibres makes the outer floss an excellent natural barrier for the cocoon. The superior abrasion resistance of degummed outer floss fibres could make fibres with good elongation, elastic recovery and work of rupture. This also could be seen from its stress-strain curve after degumming.

![SEM images of different silk fibres fractured after tensile test](image)

**Figure 2.7** SEM images of different silk fibres fractured after tensile test
In contrast to *A. pernyi* silk fibres, the greater reduction of *B. mori*, can be partly attributable to the adhesion between fibroin and sericin. As shown in Figure 2.7, compared to *A. pernyi* fibres, a clear interface between sericin and fibroin can be observed on *B. mori* fibres after fracture, indicating the interactions between the sericin and fibroin of this domestic silk are weak. Conversely, an interface between the two proteins cannot be identified for *A. pernyi* silk fibres, demonstrating strong adhesion between fibroin and sericin. The strong adhesion between sericin and fibroin of *A. pernyi* fibres could contribute to the excellent properties of the wild silkworm cocoons, protecting the worm from attack by predators or abrasion against twigs.

### 2.3.4 Dynamic mechanical properties

**Figure 2.8** displays the representative temperature dependence of the storage modulus (E’) and loss modulus (E’”) as well as loss factor tan δ of silk fibres.
Figure 2.8 Dynamic mechanical analysis of the storage modulus (a), loss modulus (b) and tan δ (c) of silk fibres from different parts. (1) Peduncle; (2) Outer floss; (3) Outermost fibre; (4) Innermost fibre

It is apparent that, the elastic modulus values ($E'$, storage modulus curve) of peduncle, outermost and innermost fibres are higher than that of outer floss fibres. Higher storage modulus is attributable to more temporary and reversible deformation along the macromolecular backbone [185]. This indicates more reversible change happens to the fibres during the variable temperature process. The reason for their high elastic modulus is most likely because of their high fibroin volume fraction. The reinforcement of fibrils imparts great strength at the interface which provides shape-preservation and stability to make its fibre very stiff and not easily deformed [186].
In the glass - rubber transition, storage modulus usually decreases due to the relaxation change of molecules. The rate of decrease, $dE'/dT$, indicates the defect level of a composite [187]. The higher the decreasing rate, the lower the defect level. The innermost and outermost fibres have higher decreasing rate (Figure 2.8 (a)), indicating they are more uniform with less defects. There is a wide temperature range for relaxation transition of outer floss (Figure 2.8 (a)), which may be due to the greater flexibility of its macromolecular chain [186].

It is worth noting that among the fibres from the different parts of *A. pernyi* cocoon, the fibres from outer floss display the earliest first onset of a decrease in $E'$ at about 100 °C, which is probably due to the molecular slippage. A combination of high content of sericin and large porosity can account for its dispersed fibril distribution and thus leads to distributed stress. This phenomenon can lead to poor thermal resistance and result in maximum weight loss ratio when exposed to high temperatures.

The maximum in loss modulus ($E''$, viscosity) is related to energy dissipation. This relaxation process involves the plasticization deformation of large molecules and is therefore associated with the glass-rubber transition of amorphous domains of silk fibre. According to ASTM E 1640 – 09, glass transition temperature is defined as the temperature of the peak in the loss modulus curves ($E''$) (See Figure 2.8b). Innermost silk, due to the higher volume fraction of fibrils (low porosity and low sericin content), has the highest $T_g$ (234.94 °C) and loss modulus ($0.77 \times 10^3$ MPa). Its broadening peak is probably due to the large content of fibroin that increases the energy adsorption. For fibres from other parts, $T_g$ showed a decreasing trend (229.59 °C, 227.65°C and 224.05°C for peduncle, outermost fibre and outer floss, respectively), whereas the maximum loss modulus corresponding to $T_g$ showed subtle changes ($0.59 \times 10^3$ MPa,
0.75 \times 10^3 \text{ MPa}, 0.54 \times 10^3 \text{ MPa for peduncle, outermost fibre and outer floss, respectively).} \ T_g \text{ value is greatly influenced by the degree of crystallinity of materials [159]. Sericin is reported to be amorphous [115]; therefore an increase in sericin content reduces} \ T_g. \text{ The negative effect of sericin on} \ T_g \text{ is more obvious when silk fibres from} \ B. \text{ mori and} \ A. \text{ pernyi} \text{ cocoons are compared (See Figure 2.9). Compared to} \ A. \text{ pernyi} \text{ fibres,} \ B. \text{ mori} \text{ fibres have a higher sericin content and their} \ T_g \text{ are about 25 °C lower, although they are less porous. This could be further confirmed by the lower} \ T_g \text{ value of} \ B. \text{ mori} \text{ innermost fibres, due to its higher sericin content compared to} \ B. \text{ mori} \text{ outermost silk fibres. For polymeric materials, the glass transition temperature is normally considered to be the temperature where polymer chain segments start to move. This value normally increases with the increasing intermolecular interactions or the presence of cross linking, while it will decrease if intermolecular interactions become weaker or the plasticization effect exits. Therefore, it can be estimated that fibres from} \ A. \text{ pernyi} \text{ has stronger bonding between sericin and fibroin than that from} \ B. \text{ mori. This will be confirmed in chapter 5.}
Tan δ, the ratio of loss modulus to storage modulus (E''/E’), indicates the damping ability of materials. Apparently, peduncle fibres have the highest tan δ, indicating their largest viscoelastic lag between the stress and the strain. This hysteresis effect can play a damping effect to reduce the amplitude of motion when a silkworm fights against foreign invasion. This may explain why peduncle fibres not only bear the weight of the silkworm cocoon but also serve to undertake the swing load rocking the cocoon back and forth in the wind. Outer floss, as the first protective barrier, has the smallest elasticity and viscosity, indicating its highest mobility and ease of deformation.
2.3.5 Hardness and elasticity

Under the applied load (5 mN), the substrate effect is negligible when the maximum penetration depth is less than approximately one tenth the fibre thickness [188].

**Figure 2.10** A schematic representation of load-displacement curves of silk fibres (a); The maximum depth of penetration and hardness of silk fibres (b). In (a), $h_t$ denotes the maximum indenter displacement and $h_f$ denotes the final depth of the residual harness impression.
Chapter 2 Microstructure and physical properties of silk fibres from *A. pernyi* cocoon

Based on the projected contact area \( A_c \) of indenter and indents, the nanoindentation hardness \( H \) and the residual modulus \( E^* \) can be derived from equations (2-3) and (2-4), respectively [189].

\[
H = \frac{P}{A_c} \quad \text{(Equation 2-1)}
\]

\[
1/E^* = \frac{(1 - \nu_s^2)}{E_s} + \frac{(1 - \nu_i^2)}{E_i} \quad \text{(Equation 2-2)}
\]

where \( P \) is the maximum load (uN); \( H \) is hardness (GPa); \( A_c \) is the contact area (nm\(^2\)); \( E^* \) is the residual modulus (GPa) integrated by silk fibre’s Young modulus \( E_s \) and indenter’s Young’s modulus \( E_i \); \( \nu_s \) and \( \nu_i \) are Poisson’s ratios of silk fibre and indenter, respectively.

As seen from **Figure 2.10**, the outermost fibres exhibit the highest lateral hardness (~0.81 GPa) but the lowest maximum penetration depth (691 nm) among all silk fibres, indicating their high defence ability against foreign physical attack. In contrast, peduncle fibres have the lowest lateral hardness (~0.22 GPa) due to their highest maximum penetration depth (1.02 um).

As the modulus \( E_i \) of the indenter (Berkovich, made of diamond) is far greater than silk fibre’s modulus \( E_s \), \( E_s \) is generally considered equivalent to \( E^* \) [190, 191]. The outermost fibres not only have the highest hardness, but also possess the best elasticity (~14.60 GPa), indicating their excellent capacity of anti-deformation. The superior hardness and elastic modulus contribute to their protection for pupa residing inside. In contrast, peduncle fibres with the lowest elasticity (~10.25 GPa) could be a buffer material. That helps to dissipate collision energy and reduce damage, which is consistent with the results of DMA in the longitudinal direction. This can be interpreted by the *in-situ* optical micrograph of a linear array of indents made on fibres (**Figure 2.11**). The peduncle and innermost fibres are severely damaged but the fibres from the outermost layer have very slight imprints of indentation stylus due to their...
good elasticity. Outermost and innermost fibres from *B. mori*, have nearly the same performance as innermost fibres from *A. pernyi*.

![Figure 2.11](image)

**Figure 2.11** Optical micrographs of silk fibre surface with a line of indents. (a) A. peduncle; (b) A. outer floss; (c) A. outermost fibre; (d) A. innermost fibre; (e) B. outermost; (f) B. inner.

### 2.4 Conclusions

In summary, silk fibres from three key parts of the *A. pernyi* silkworm, i.e. peduncle, outer floss and cocoon shell (both outermost and innermost parts) were studied in this work. Fibres from the different parts have different microstructure and physical properties, leading to various protective functions. Peduncle fibres showed well aligned arrangement, good plastic deformation and superior hysteresis effect to other fibres, which effectively reduce the amplitude of swinging back and forth when it
resists harsh weather conditions like strong wind. Because of the high sericin content, the outer floss has many desirable physiological properties, such as anti-oxidant and UV protection. The outermost fibres have good hardness and elasticity, leading to their remarkable toughness to absorb the most breaking energy. Innermost fibres have superior elasticity therefore is shape-preservation and stable. Consequently, fibres from different parts of the *A. pernyi* cocoon have a combination of hysteresis, toughness as well as shape-preservation, which facilitates the protection of silkworms in the wild.

Silk fibre is formed by protein molecules with ordered supramolecular nanostructures, $\beta$-sheet crystallites and amorphous chains. The hierarchical structure of silk determines its mechanical properties, which will be investigated in more detail in chapter 3.
CHAPTER 3 Correlating mechanical properties of *A. pernyi* silk fibres to crystalline nanostructures

3.1 Introduction

Chapter 2 gives information about the relationship between the structure and mechanical properties of fibres from different parts of the *A. pernyi* cocoon. Their correspondingly desirable functions are derived from their repetitive peptide sequences that fold into regular structural elements. This realization has inspired many explorations into the use of well-defined secondary structural motifs to control the structure and properties of protein-based synthetic polymers [109, 192, 193].

The impressive mechanical properties of silkworm and spider silks are due to their nanocrystalline networks which form ordered regions of β-sheets, with numerous inter- and intramolecular hydrogen bonds, and disordered regions [193]. The intermolecular β-sheet structures impart extraordinary strength, while amorphous peptide regions contribute to the elasticity and toughness of silk fibres [194]. The intramolecular β-sheet structures play a minor role in determining the strength, toughness, and elasticity of silk fibres [195]. These indicate that by manipulating the amount and ratio of different motifs, one could prepare protein structures with tuneable mechanical properties. However, despite the significant efforts that have been devoted to mimicking the natural silk spinning process, silk fibres produced artificially lack sufficient mechanical robustness [196]. For example, the tensile failure stress of native *B. mori* silk fibre is 590 MPa, but it is reduced to about 60 and 10 MPa, respectively, for artificially drawn and undrawn fibres [197]. In this context, understanding the
influence of nanocrystalline structure of silk fibres on their mechanical properties is significant to develop artificial protein fibres with superior mechanical properties. Varying the nanocrystalline structure of natural silk fibres and understanding its effect on their mechanical properties is the most convenient way to achieve this objective.

It has been demonstrated that thermal treatment could be a simple and effective approach to tune the mechanical properties of silk fibres. For example, Shao et al. reported that by cooling to -60 °C, silk fibres with increased strength and doubled elongation to fracture could be produced [120]. However, a basic and quantitative understanding of the temperature influence on the nanocrystalline structure and hence the mechanical properties of silk fibres has not been obtained. Although variation of crystallite size of spider silk during thermal treatment has been reported recently, how the change in crystallite size affects the mechanical properties of the silk fibres remains to be understood [143].

Thermal treatments are important for many technical processes, for example sintering, annealing, oxidization and carbonisation, to fabricate high-performance materials [198]. Therefore, it is important to evaluate how the nanostructure variations due to thermal treatment affect the physical performance of the silk fibres.

In this chapter, fibres from different parts of the A. pernyi cocoon and B. mori cocoon were annealed at different temperatures. The changes in the nanocrystallite structure and mechanical properties of the fibres were monitored. The mechanical performance of different silk fibres was examined by tensile test, DMA analysis, and repeated bending/abrasion fatigue (FibreStress) test. FTIR and XRD were used to analyse the secondary structure and crystallite size of different silk fibres. The findings obtained
here could shed light on our understanding of silkworm silk and provide insight into the design of functional materials with desired physical properties.

3.2 Experimental

3.2.1 Silk fibre preparation

The details on silk degumming have been given in chapter 2, section 2.2.1.

The degummed silk fibres are thermally-heated under anaerobic conditions (N$_2$) up at 50, 100, 150, 230 °C, respectively for 15 min.

3.2.2 Fourier Transform Infrared Spectroscopy (FTIR)

ATR-FTIR measurements are performed to study the secondary structure of silk fibres after silk degumming. The samples are scanned from 4000 cm$^{-1}$ to 600 cm$^{-1}$ at a rate of 4 cm$^{-1}$/min, and 64 scans are averaged for each sample. The background spectra are collected under the same conditions and subtracted from the scans for each sample. Fourier self-deconvolution (FSD) of the infrared spectra covering the amide IR region (1590-1710 cm$^{-1}$) is performed with Origin 9.0 software to examine the amount of each phase in silk fibres.

3.2.3 Wide Angle X-ray Diffraction (WAXRD)

The X-ray diffraction pattern of silk fibres are performed on a PANalytical’s X’Pert Powder machine with Cu Kα radiation ($\lambda = 0.154$ nm) at 40 kV and 30 mA in the angle range of 5 - 40° (2θ). The scanning rate is 0.02 °/min with a spot size of 10 mm $\times$ 10 mm. Deconvolution of the WAXRD profiles is performed using Gaussian (Origin 9.0, See Figure 3.1). The Bragg reflections are separated from the broad short-range order background to estimate the crystalline area to total area (crystallinity C) (Equation 3-
1) The crystallite size \((L)\) along axis is determined by the Scherrer equation (Equation 3-2). The position and FWHM of these peaks are used to determine the crystallite size along the \(\vec{a}, \vec{b}\) and \(\vec{c}\) axes, with \(d\)-spacing expressed for the orthorhombic lattice as a function of the lattice parameters (Equation 3-4).

\[
C = \frac{A_c}{A_a + A_c} \times 100\% \quad \text{(Equation 3-1)}
\]

\[
L = \frac{k\lambda}{FWHM_{\text{coss}}} \quad \text{(Equation 3-2)}
\]

\[
2d\sin\theta = \lambda \quad \text{(Equation 3-3)}
\]

\[
\frac{1}{d} = \sqrt{\left(\frac{h}{a}\right)^2 + \left(\frac{k}{b}\right)^2 + \left(\frac{l}{c}\right)^2} \quad \text{(Equation 3-4)}
\]

where \(A_c\) is the area under the crystalline diffraction peaks and \(A_a\) is the area of amorphous zone; \(k\) is Scherrer constant, 0.9; \(\lambda\) is the diffractive wavelength \((\lambda = 0.154 \text{ nm})\); FWHM(hkl) is the half width of diffraction peak; \(\theta\) is the scattering angle; \(d\) is the lattice spacing; \(a, b\) and \(c\) are the lattice constants.

3.2.4 Temperature-dependent mechanical properties

The details have been given in chapter 2, section 2.2.7.

3.2.5 Tension tests of silk fibres

The details have been given in chapter 2, section 2.2.6.

3.2.6 Nano-indentation test

The details have been given in chapter 2, section 2.2.8.
3.3 Results and discussion

3.3.1 Hierarchical structure of silk fibres

To determine the conformational structure of silk fibres from their IR spectra, the amide I region between 1590 and 1710 cm\(^{-1}\), assigned to the peptide backbone, was focused on. Various conformations of silk secondary structures (random coil, helices and β-sheet) have characteristic bands in the infra-red spectrum. In general, the amide I mode is associated with the α-helical conformation at 1650-1660 cm\(^{-1}\); the random coil conformation gives bands in the range of 1640-1650 cm\(^{-1}\); the β-sheet conformation results in IR bands between 1610 and 1635 cm\(^{-1}\); the β-turn structures show at 1666-1685 cm\(^{-1}\); and the Tyr side chains presents at about 1604 cm\(^{-1}\). Figure 3.1 gives a comparison of structural characterizations of silk fibres annealed at different temperatures.
Figure 3.1 ATR-FTIR spectra of silk fibres annealed at different temperatures. If not specified, peduncle, outer floss, outermost and innermost fibres are from *A. pernyi*.

The spectra above were respectively fitted into ten peaks for various secondary structures including random coils at 1647 cm$^{-1}$, helical conformation at 1659 cm$^{-1}$, β-turns at 1666, 1678 and 1685 cm$^{-1}$, β-sheets at 1616, 1624, 1635 and 1697 cm$^{-1}$, and (Tyr) side chains at 1604 cm$^{-1}$. (a) and (b) are the examples of curve fitting for innermost fibres annealed at 25° (a) and 230° (b), respectively.

As temperature increases, the peaks at about 1658 cm$^{-1}$ and 1650 cm$^{-1}$ disappear, indicating the content of α-helical structure decreases; meanwhile the peak at 1627 cm$^{-1}$...
slightly shifts to 1622 cm\(^{-1}\) with a sharper contour, demonstrating more \(\beta\)-sheets forms.

The fractions of helices, \(\beta\)-sheets, and random coils in the fibres were estimated by adding the areas of all bands assigned to each of these structures covering the Amide I region. The results are plotted against annealing temperatures in Figure 3.2.

**Figure 3.2** The fractions of helices, \(\beta\)-sheets (\(\beta\)), and random coils in silk fibres as a function of annealing temperatures
The overall $\beta$-sheet contents of all the fibres from *A. pernyi* increase significantly when the temperature is increased from room temperature to 50 °C, which is accompanied by a sharp decrease in the contents of random coils. At temperatures above 50 °C, the content of $\beta$-sheets slightly drops, then increases again dramatically when the temperature is above 150 °C, the contents of random coils gradually decreases. The fraction of helices is nearly independent of the annealing temperature, except for a big change at about 100 °C. The content of $\beta$ sheets reached its highest at the annealing temperature of 230 °C. The transformation to the $\beta$-sheet structure occurs mostly due to the conformational transition from random coil to $\beta$-sheet, whereas the fraction of helices changes independent of increasing temperature with irregular change. Hence, the maximum in the $\beta$-sheet content is accompanied with a minimum in the content of random coils.

*B. mori*, on the contrary, shows a steady increase in the $\beta$-sheet content up to the highest annealing temperature of 230 °C. A reduction of random coil fraction of this silk is accompanied with an obvious increase in its $\beta$-sheet and helices contents, indicating a conformational transition from random coil to $\beta$-sheet structure.

With heating, silk protein undergoes a dramatic refolding to convert to a $\beta$-sheet enriched state. This $\beta$-sheet enriched state remains stable upon returning to ambient temperature and is hence irreversible. This means energy is required to give rise to an enriched $\beta$-sheet state. Compared to *B. mori*, *A. pernyi* silk shows an abrupt increase of $\beta$-sheet content at 50 °C, indicating even at a low temperature, *A. pernyi*, especially the outermost fibre (with 12 % increase in $\beta$-sheet content), is more susceptible to thermal treatment for secondary conversion.
3.3.2 Determination of the size of the β-sheet crystalline regions

The XRD patterns of *A. pernyi* silk were determined with Brag reflections of (020), (210), (002) and (230) peaks for β-sheet structure. *B. mori* silk however showed identical β-sheet crystalline reflections of (100), (200), (120) and (002) peaks, respectively. Figure 3.3 shows XRD diffractions with typical crystalline reflections for *B. mori* and *A. pernyi*, respectively.

**Figure 3.3** Scattering profiles with Brag reflections for *B. mori* and *A. pernyi* (outer floss) silk fibres

To calculate the crystallinity (C %) and the crystallite size (L) for different fibres, deconvolution of each profile was performed and the results are listed in Table 3.1.
Table 3.1 Crystallinity and crystal size of different silk fibres

<table>
<thead>
<tr>
<th>Silk fibres</th>
<th>Annealing temperature / °C</th>
<th>Overall content of β-sheet, %</th>
<th>Intra-molecular β-sheet, Crystallinity / %</th>
<th>Intra-molecule β-sheet, %</th>
<th>FWHM / °</th>
<th>Crystallite size / nm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>L  a  b  c</td>
</tr>
<tr>
<td>Peduncle</td>
<td>25</td>
<td>56.74 (100%)</td>
<td>54.40(95.87%)</td>
<td>2.34 (4.13%)</td>
<td>2.61</td>
<td>3.09 5.54 5.04 9.01</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>60.47 (100%)</td>
<td>52.07(86.11%)</td>
<td>8.4 (13.89%)</td>
<td>2.50</td>
<td>3.22 6.51 5.84 6.91</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>59.47 (100%)</td>
<td>51.91(87.29%)</td>
<td>7.56 (12.71%)</td>
<td>2.48</td>
<td>3.26 7.25 6.07 7.40</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>61.33 (100%)</td>
<td>51.86(84.56%)</td>
<td>9.47(15.44%)</td>
<td>2.46</td>
<td>3.27 7.46 5.58 7.39</td>
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<tr>
<td></td>
<td>230</td>
<td>64.12 (100%)</td>
<td>50.73(79.12%)</td>
<td>13.39(20.88%)</td>
<td>2.30</td>
<td>4.06 7.97 5.87 8.95</td>
</tr>
<tr>
<td>Outer floss</td>
<td>25</td>
<td>56.62 (100%)</td>
<td>51.98(91.81%)</td>
<td>4.64(8.19%)</td>
<td>2.33</td>
<td>3.46 6.14 5.74 6.52</td>
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<tr>
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<td>50</td>
<td>58.35 (100%)</td>
<td>50.19(86.01%)</td>
<td>8.16(13.98%)</td>
<td>2.11</td>
<td>3.82 6.93 6.13 6.38</td>
</tr>
<tr>
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<td>100</td>
<td>56.51 (100%)</td>
<td>48.34(85.54%)</td>
<td>8.17(14.45%)</td>
<td>2.06</td>
<td>3.91 7.44 5.90 6.31</td>
</tr>
<tr>
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<td>56.97 (100%)</td>
<td>44.96(78.92%)</td>
<td>12.01(21.08%)</td>
<td>2.02</td>
<td>3.99 7.58 6.00 6.02</td>
</tr>
<tr>
<td></td>
<td>230</td>
<td>65.01 (100%)</td>
<td>44.21(68%)</td>
<td>20.8(32%)</td>
<td>1.96</td>
<td>4.11 7.31 6.17 6.29</td>
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<tr>
<td>Outermost</td>
<td>25</td>
<td>55.47 (100%)</td>
<td>52.68(94.97%)</td>
<td>2.79(5.03%)</td>
<td>2.26</td>
<td>3.58 6.68 5.48 9.10</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>62.07 (100%)</td>
<td>51.06(82.26%)</td>
<td>11.01(17.74%)</td>
<td>2.23</td>
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<td>8.79(15.04%)</td>
<td>2.15</td>
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<tr>
<td></td>
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<td>45.79(77.81%)</td>
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<td>2.02</td>
<td>3.99 7.82 5.77 8.63</td>
</tr>
<tr>
<td></td>
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<td>62.82 (100%)</td>
<td>45.67(72.7%)</td>
<td>17.15(27.3%)</td>
<td>1.93</td>
<td>4.17 9.45 5.34 9.55</td>
</tr>
<tr>
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<td>57.97 (100%)</td>
<td>55.75(96.17%)</td>
<td>2.22(3.83%)</td>
<td>2.28</td>
<td>3.54 6.72 5.30 9.14</td>
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<tr>
<td></td>
<td>50</td>
<td>57.77 (100%)</td>
<td>53.63(92.83%)</td>
<td>4.14(7.17%)</td>
<td>2.02</td>
<td>3.99 7.78 5.81 7.56</td>
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<tr>
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<td>58.58 (100%)</td>
<td>52.06(88.87%)</td>
<td>6.52(11.13%)</td>
<td>1.84</td>
<td>4.14 8.89 6.16 7.30</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>61.13 (100%)</td>
<td>51.82(84.77%)</td>
<td>9.31(15.23%)</td>
<td>1.89</td>
<td>4.26 9.19 6.26 7.50</td>
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<tr>
<td></td>
<td>230</td>
<td>66.59 (100%)</td>
<td>49.87(74.89%)</td>
<td>16.72(25.11%)</td>
<td>1.87</td>
<td>4.31 9.36 5.67 8.06</td>
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<td>56.89(96.52%)</td>
<td>2.05(3.48%)</td>
<td>3.18</td>
<td>2.54 4.56 5.26 6.10</td>
</tr>
<tr>
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<td>52.75(88.31%)</td>
<td>6.98(11.69%)</td>
<td>2.94</td>
<td>2.75 4.63 4.84 5.89</td>
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<tr>
<td>Innermost</td>
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<td>60.95 (100%)</td>
<td>47.86(78.52%)</td>
<td>13.09(21.48%)</td>
<td>2.74</td>
<td>2.95 4.88 4.70 5.42</td>
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<tr>
<td>Innermost</td>
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<td>61.48 (100%)</td>
<td>45.72(74.37%)</td>
<td>15.76(25.63%)</td>
<td>2.87</td>
<td>2.82 5.34 4.45 5.41</td>
</tr>
<tr>
<td>Innermost</td>
<td>230</td>
<td>60.15 (100%)</td>
<td>38.06(63.28%)</td>
<td>22.09(36.72%)</td>
<td>2.58</td>
<td>3.13 6.07 5.17 5.62</td>
</tr>
</tbody>
</table>

[a] Intra-molecule β-sheet (%) = Overall content of β-sheet (%) – Crystallinity (%)
There are two types of β-sheets in silk fibres: the intra-molecular β sheets (non-crystalline structure) and inter-molecular β sheets (crystallites) [199]. The contents of overall β-sheets and the inter-molecular β-sheets can be identified by FTIR and XRD, respectively [199]. The percentage of intra-molecule β-sheets can be obtained by deducing β-crystallites from the overall content of β-sheets. Table 3.1 shows that for fibres from both types of cocoons, increasing temperature, the fraction of crystalline β-sheets is generally reduced, whereas that of the non-crystalline β-sheets is increased.

The overall increasing tendency of intramolecular β-sheet indicated that, the polypeptide chain could bend onto itself to form parallel β-sheets due to heat treatment. The formation of parallel β-sheet through intramolecular hydrogen bonding in peptides linked by flexible linear chains was entropically promoted. The long peptide segments have the tendency to self-assemble into antiparallel/intermolecular β-sheet aggregates, whilst short alanine-based peptides, such as (Ala)$_3$, were found to organise into parallel β-sheets [200].
It can be concluded from Table 3.1 that, *B. mori* silk fibres have higher crystallinity, higher orientation [112] and smaller crystallite size, whereas *A. pernyi* fibres show lower crystallinity, lower orientation and bigger crystallite size. This would lead to superior stress of *B. mori* silk fibres. However, annealing the *B. mori* fibres at 230 °C reduces its crystallinity significantly (See Table 3.1), which may have an impact on the mechanical properties of the fibres.

The crystalline fraction of silk fibroin consists of anti-parallel pleated β-sheets of polypeptide chains packed into orthorhombic unit cells [60]. Stacked β-sheets with peptide chains connected by hydrogen bonds constitute the β-crystallite as shown in Figure 3.4. The lattice constants of the β-crystallites are *a* (the inter-sheet direction
with van der Waals force), b (the inter-chain direction with hydrogen bonding) and c (fibre axis with covalent force). As shown in Table 3.1, with the increase of temperature, the crystallite of both A. pernyi and B. mori in c direction becomes small, whereas in a and b directions it becomes bigger, indicating the crystallite undergoes thermal contraction.

Compared to B. mori, a greater reduction of crystallite size happened to A. pernyi fibres in c axis with an increase in temperature (See Table 3.1). This indicates that more shrinkage occurred in A. pernyi fibres. In comparison, the crystallite size in b and c directions changes very little, due to the strong hydrogen bonds between amide linkages of the adjacent chains and the peptide linkage between peptides.

A comparison of the crystal lattices of different fibres from the A. pernyi cocoon revealed that outer floss fibre has a larger ab plane and a shorter c plane. The difference in the ab plane indicates the different intermolecular interactions of poly(alanine) sequences among A. pernyi silk fibres. The longer linear hydrogen bonds between antiparallel β-strands are not stable and prone to thermal contraction. Similarly, with the increase of temperature, the significant increase in ab plane of crystallites of outermost fibres (See Table 3.1) indicates these fibres would have marked thermal shrinkage than innermost fibres.

Table 3.1 also shows that increasing the thermal annealing temperature, the crystallinity of all the fibres decreased. The inter-sheet distance and crystallites show an increased coherence length along the a (200) direction and the β-sheet (210) reflection as compared to the fibre axis (002) direction. Two factors may account for this phenomenon. First, the macropores in A. pernyi silk provide enough space for crystal growth during heat treatment [77]. Therefore, the crystallite grows along a
preferred direction, namely, orientation of pore channel. Second, heat treatment would produce defects in the changing process of crystals, thus lowering the orientation $[112]$ and crystallinity of fibres.

### 3.3.3 Dynamic mechanical thermal analysis

To analyse in detail how thermal treatment affects viscoelasticity of fibres, single filaments were tested on DMA. A representative scan of the dynamic mechanical properties of silks is displayed in Figure 3.5.

![Figure 3.5](image)

**Figure 3.5** Representative dynamic mechanical curves of silk fibres. (a) Storage modulus; (b) Loss tan δ
In Figure 3.5(a), the curves of silk fibres show the typical thermoplastic behaviour. *A. pernyi* silk fibres were in a glassy state at temperatures below 189 °C and in a rubbery state, in which the amorphous chains entangled together, when temperature is above 265 °C. *B. mori* fibres are in a glassy state at temperatures below 160 °C and in a rubbery state when temperature is above 235 °C. Within the glassy state, *B. mori* shows the highest storage modulus due to the stronger intermolecular interactions. Among *A. pernyi* silk fibres, the innermost fibre shows the highest storage modulus followed by outermost fibre in glassy and rubbery state, indicating strongest ability to resist intermolecular slippage. This may be correlated to their superior crystallinities. The storage modulus drops quickly when the temperature is above a threshold, which is about 160 °C and 189 °C for *B. mori* and *A. pernyi* silk fibres, respectively. The dramatic decrease of storage modulus corresponds to the primary relaxation associated with the glass-rubber transition of amorphous silk. It is due to the energy dissipation where the loss tan δ passed through a maximum.

There are two peaks in loss tan δ curves for *A. pernyi* silks, but only one for *B. mori*. Compared to the peak at 189 °C for *B. mori*, the first peak shown at 230 °C for peduncle, 224 °C for outer floss, 228 °C for outermost silk, and 235 °C for innermost silk, was attributable to $T_\alpha$ (Figure 3.5), which is the critical temperature to breakdown the intermolecular hydrogen bonds in the non-crystalline segments. The higher value of $T_\alpha$, indicates *A. pernyi* silk fibres, especially the innermost silk, may retain quite exceptional mechanical properties over a temperature range. The distinct small peaks for *A. pernyi* in the range of 289-300 °C (peduncle 289 °C, outer floss 291 °C, outermost 300 °C and innermost 290 °C) (Figure 3.5(a)), are also accompanied by increasing storage modulus (Figure 3.5(b)), indicating *A. pernyi* silk fibres become stiffer which may affect its physical property. The transition in this process can be
attributable to the partial crystallisation due to the improved crystalline structure [201]. On the other hand, the relatively large altitude ($I_\alpha$) of loss tan δ peak at $\alpha$ relaxation temperature ($T_\alpha$) implied that compared to $B. mori$, $A. pernyi$ had more mobile units participating in the relaxation process. This may imply $B. mori$ fibre has relatively higher orientation in amorphous region, which needs more energy to move.

The slight increase shown in Figure 3.5(b) at around 50 °C for $A. pernyi$ silks is attributed to secondary relaxation. It indicates an increase in the intrinsic stiffness of individual molecular chains with some rearrangement of the molecules in the amorphous regions during heat treatment [112]. This may account for the abrupt increase of $\beta$-sheet structure at 50 °C shown in Figure 3.2.

Significant differences in cumulative shrinkages of the various $A. pernyi$ silk fibres and $B. mori$ silk fibres upon thermal treatment were detected (Figure 3.6). Large contractions of 5% can be induced at 230 °C. Thermal contraction is typically associated with entropic elasticity.
 Compared to *B. mori* silk fibres, the marked shrinkage behaviour is very characteristic of *A. pernyi* silk fibres. *A. pernyi* fibres shrink lengthwise dramatically when exposed to low temperatures under relaxed conditions. This entropy shrinkage is a function of molecular heat relaxation. Macroscopic shrinkage along the fibre axis is primarily an entropy-driven process, with ‘chemical’ effects serving to modify the entropic response. These findings suggested that there should be some rearrangements of the *A. pernyi* fibroin molecules in the amorphous regions during the heating process, resulting in a strengthening of the intrachain interactions. *B. mori* showed good thermal stability at temperatures below 150 °C, partly attributed to the higher degree of molecular orientation even in the amorphous regions as well as to the more compact fibrous structure. However, *B. mori* fibres dramatically contract above 150 °C. The dramatic shrinkage is probably due to melting of the β-pleated sheet crystals, removing
the physical crosslinks. At a high temperature, it is noticeable that less shrinkage occurs to *A. pernyi* silk fibres. This is probably because of the presence of a greater degree of residual stress in these fibres.

### 3.3.4 Tensile strength changes induced by thermal annealing

Silk can be considered as protein elastomers [202]. This is shown by the temperature-dependent stress-strain curves of degummed silk fibres from different parts of the *A. pernyi* cocoon (Figure 3.7). In the early region of the curves (Figure 3.7), the silk fibres fulfil the Hooke’s Law at all temperatures. The stress and strain of different fibres as a function of temperature are summarised in Figure 3.8 (a) and (b), respectively. Young’s modulus and yield strength versus temperature are shown in Figure 3.8 (c) and (d).
Figure 3.7 Representative stress-strain curves of different silk fibres

Figure 3.8 summarises the mechanical properties of different silk fibres annealed at temperatures in the range of 0-250 °C. It is noted that, both strain-to-failure and stress decreased along with an increase in temperature while Young’s modulus continues to rise with temperature up to 230 °C (Figure 3.8 (c)). The origin of these changes has to be located in the decrease amorphous chain fraction as well as chain scission from its crystalline structure.
Figure 3.8 Evolution of mechanical properties of different silk fibres after thermal treatment

Figure 3.8 (c) shows that the Young’s modulus increases from about 3.8 MPa at 25 °C to around 8 MPa at 230 °C. As discussed in chapter 1, at small tensile strains, the intramolecular β-sheets within the amorphous regions unfold to release the length of protein chains, while the β-crystallites remain unaffected. Therefore, Young’s modulus is proportional to the strength of intramolecular β-sheets and therefore increases along with the increase of temperature.

Yield strength is the stress at which plastic deformation becomes noticeable and significant. As shown in Figure 3.8 (d), thermal treatment improves the yield strength ($\sigma_y$) of silk fibres. Compared to B. mori, the yield strength of A. pernyi silk fibres is more influenced by temperature. The yield strength of A. pernyi silk fibres is increased
by almost 60%, whilst the same heat treatment only lead to about an increase of 10% in \( \sigma_y \) from 100 MPa at 25 °C to 110 MPa at 230 °C for \( B. \) mori fibres. The significant increase in \( \sigma_y \) indicates thermal treatment can stiffen \( A. \) pernyi silk fibres, making the fibre more resistant to further deformation. It also led to the remarkable decrease in strain of \( A. \) pernyi silk fibres, as toughness decreases due to plasticization. Interestingly, the variation of \( \sigma_y \) versus crystallite size is a positive relation, which explains the inverse Hall-Petch relation [203]. This is probably because when the crystallite size is below 10 nm, the yield strength is mainly determined by the crystallite size. This could be further confirmed by Table 3.1 and Figure 3.8 (d), that the innermost fibre, followed by outermost fibre, peduncle and outer floss fibres from \( A. \) pernyi shows the highest yield strength due to its biggest crystallite size and \( \sigma_y \) also tends to increase with increasing crystallite size.

The decrease of breaking extension is originally generated from the decrease of amorphous volume fraction (See Figure 3.2). The reduction in both strength and stiffness caused by heat treatment is mainly attributed to the insulation and destruction of intermolecular chains, because silk fibres undergo changes in atomic and crystalline structures at this stage. This stage is assumed to produce the end groups with –NH\(_2\) and –OH, which would react with its neighbouring chains. The increase of these groups with defects would reduce the stress and strain to failure.

3.3.5 Nano-hardness

Nano-indentation tests were performed to examine the effect of annealing treatment on hardness and elastic modulus on a reduced length scale. Figure 3.9 shows the representative load-depth curves of silk fibres as well as hardness and elastic moduli and the results are summarised in Figure 3.10.
Figure 3.9 Typical load-depth curves of different silk fibres at different annealing temperatures
Figure 3.10 Hardness and elastic moduli obtained from nanoindentation tests as well as the relationship between hardness and yield strength for silk fibres. (a) the maximum penetration depth; (b) elastic moduli and (c) hardness obtained from nanoindentation tests of silk fibres; (d) the relationship between hardness and yield strength for silk fibres.

With an increase in the temperature for thermal treatment, the elastic moduli of all the silk fibres decreased, while their hardness increased. Its hardness was increased from only 0.02 GPa at 25 °C to about 0.15 GPa at 230 °C (Figure 3.10(c)) and its elastic modulus was decreased from about 5 GPa at 25 °C to only 0.5 GPa at 230 °C (Figure 3.10(b)).

Increasing temperature results in an increase in hardness of silk fibre. The increase of hardness is more prominent for A. pernyi fibres. This significant enhancement in
hardness is primarily attributed to the confinement effects of oriented intra-molecular networks, restricting the movements and folding of the polymer chains. Consistent with Table 3.1, outer floss has the highest intramolecular β-sheet at 230 °C, whereas peduncle has the lowest content of intramolecular network at this temperature, which contribute to their highest hardness and lowest hardness (Figure 3.10 (c)), respectively. The hardness of B. mori fibre also increases with annealing temperature. However, it is lower than that of A. pernyi fibres. This is probably because of the different repetitive peptide unit in the crystalline network.

More importantly, the hardness of silk fibres shows a linear relationship with yield strength (Figure 3.10 (d)), slightly deviating from the Tabor’s empirical relationship, $H = C\sigma_y$. This deviation is due to the fact that polymeric materials exhibit elastic and plastic deformation under tension, namely the viscoelastic behaviour.

### 3.4 Conclusions

In this chapter, the crystalline structure of A. pernyi and B. mori silk fibres was tuned by thermal treatments and influences of thermal treatments on the mechanical properties of the fibres were investigated. Thermal treatment had a significant impact on the nanostructure formation of silk fibres. It can lower the tendency to self-assemble into β-crystallite but promote the formation of intramolecular β-sheet. The temperature-induced structural changes affect the mechanical properties of the fibres. Tensile measurements suggested that mechanical properties could be modulated by annealing the building blocks. The stress and strain to failure decreased with increasing temperature and reached a minimum at around 230 °C, whilst modulus and yield strength increased with temperature. Compared to B. mori, A. pernyi silk fibre has more obvious temperature-dependant structural changes. In the elastic region, the
intramolecular β-sheet plays a leading role in modulating the elastic modulus and hardness, while the crystallite size has a positive effect on the yield strength. In contrast, the intermolecular β-sheet decides the breaking tensile strength. The outermost and innermost fibres from *A. pernyi* show overall superior mechanical properties in stress, strain, modulus, yield strength, hardness and elasticity.

In summary, the observations of this chapter demonstrate that the mechanical properties of silk fibres can be tuned by manipulating their crystalline nanostructures. Thermal treatment is a feasible and simple approach for this purpose. The observations will be helpful to the design of protein/polymer materials with desirable properties of different applications.
CHAPTER 4 Thermal conductivity of A. penryi silk fibres

4.1 Introduction

As explained in the literature review, silk cocoon shells exhibit a high level of thermal buffer and damping. Its thermal conductivity ($k$) is about 0.05 W/m$\cdot$K which is very close to that of silk fabrics (0.042 W/m$\cdot$K) in the thickness direction [65, 133]. The low thermal conductivity is mostly because of strong phonon scattering by various defects and interfaces in silk fibres [135]. Generally, electrical insulation materials, like fibres, are phonon-based conductors of heat [204], in which, the phonons are mostly scattered by crystallite boundaries [205].

Significant interest has been shown on the thermal properties of single silk fibres. The results indicate that by improving the crystalline structure, for example, by stretching, the thermal conductivity of the fibres can be enhanced. For instance, Wang et al. reported that, the thermal diffusivity of spider silk varies from $8.38 \times 10^{-5}$ to $12.30 \times 10^{-5}$ m$^2$/s (46.8 % increase) and thermal conductivity $k$ is from 348.7 to 415.9 W/m$\cdot$K (19.3% increase) when strain was increased from 3.9% to 19.7%, respectively [8]. In contrast, thermal conductivity and diffusivity of single B. mori silkworm silk are 0.39 – 2.03 $\times$ 10$^{-6}$ m$^2$/s and 0.54 – 6.53 W/m$\cdot$K, respectively, upon elongation up to 63.8% [128]. Despite of these interesting observations, the reliability of results is questionable since the reported thermal conductivity/diffusivity values of individual single fibres vary by one order of magnitude. In addition, it is impossible to compare the thermal properties of different fibres on the basis of literature reports, since different techniques were used for the fibres in different forms. Furthermore, the crystal structure was not adequately characterised in the reports and consequently, the
structure-thermal property relationship could not be established. Deciphering the correlation between the thermal property and the crystallite may improve our fundamental understanding of the nanoscale thermal transport phenomena in biomaterials. This may further guide the design of synthetic silk materials with tuneable thermal properties. Though amorphous phase also plays a role in determining the thermal property of silks, compared with β-sheets, the amorphous phase is typically associated with more phonon scattering that reduces thermal conduction. Therefore, the β-sheets, which are main thermal conductors in silks, will be the focus in this study to reveal the effect of the crystallite on their thermal properties.

In this chapter, different layers of *A. pernyi* cocoon are tested to quantify the thermal conductivity/diffusivity, the corresponding degummed silk fibres are tested in order to simulate the thermal conductivity/diffusivity of single fibres. A structural model is established to explain the observed thermal diffusivity and the thermal path breakdown. Also, the effects of crystalline structure and porosity on the phonon thermal conductivity of silk fibres are analysed.

### 4.2 Experimental

#### 4.2.1 Sampling preparation

For measuring the thermal diffusivity of silk cocoon, different raw silk layers from *A. pernyi* and *B. mori* are cut into circular disks with a diameter of 13 mm. To analyse the contribution of individual parts of silk cocoons, the thermal diffusivities of sericin powder and fibroin fibres as well as calcium oxalate powders (deposits on *A. pernyi* cocoon) are examined. Sericin powder obtained from degumming silk fibres is pressed into pellets with a diameter of 13 mm under a pressure of 10 tons. To measure the
thermal diffusivity in the transverse direction, the degummed silk fibres are aligned into a pellet with a diameter of 13 mm under a pressure of 10 tons. Commercial calcium oxalate crystals are also pressed into pellets with the same method.

To prepare sericin proteins, a base degumming method is used to obtain sericin from both the *A. pernyi* and *B. mori* silk. Briefly, a mixed solution of 1% base (Na2CO3 plus NaHCO3 in equal weight proportion) at 98 °C is used to degum *A. pernyi* cocoons (1h) as well as *B. mori* cocoons (30 mins). The degummed fibres are thoroughly rinsed with warm DI water (60 °C) prior to being dried in air. The sericin solutions obtained from degumming are dialyzed against DI water using snakeskin pleated dialysis tubing for 3 days to remove salt ions. The dialysate is then centrifuged at 7000 rpm and 4 °C for 20 min to remove impurities and aggregates. The sericin solutions are further freeze dried to obtain sericin powders.

To measure the thermal diffusivity in the longitudinal direction, a bundle of silk fibres, is held with polyimide tape and cut into a cylinder with a length of 3 mm (Figure 4.1). To compare the thermal diffusivities of silk fibres with other natural fibres, samples of other fibres, including wool (Australian wool, average width: 13.46 ± 0.69 μm), cotton (Australian cotton, average width: 18.15 ± 0.32 μm) and ramie (China, average width: 29.74 ± 5.79 μm), are also prepared.

The regenerated *A. pernyi* fibroin films are obtained by degumming silk fibres, dissolving fibroin fibres (LiSCN (10 M) at 55 °C for 2 h), and desalinating by dialysis prior to casting to form a fibroin film. To illustrate the effects of crystallinity or the density of crystallites on thermal diffusivities, the films were further crystallized with 80% aqueous methanol solutions for 5 and 60 min in order to get films with a gradient conformational changes.
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Calcium oxalate crystals were purchased from Sigma-Aldrich and used as received.

![Figure 4.1 Examples of sample preparation of fibre pellet (a), bundles (b), films (c) and powders (d)](image)

**Figure 4.1** Examples of sample preparation of fibre pellet (a), bundles (b), films (c) and powders (d)

4.2.2 Reagents

Sodium carbonate (Na$_2$CO$_3$), sodium bicarbonate (NaHCO$_3$), calcium chloride (CaCl$_2$), ethyl alcohol (EtOH), lithium thiocyanate (LiSCN) and sodium hydroxide (NaOH) were all purchased from Sigma Aldrich (Australia) and are of analytical grade with a purity of at least 98%. Dialysis tubing cellulose membrane (12kDa) was purchased from Sigma-Aldrich, USA. Snakeskin pleated dialysis tubing (3.5kDa) was
purchased from Thermo Fischer Scientific, Australia. Deionised (DI) water was used throughout the study.

4.2.3 Density

Fibre density is tested using an Automatic Density Analyzer (Ultrapycnometer, Quantachrome Instrument). The densities of fibroin films are calculated using weight-volume method. Figure 4.2 shows the sample preparation and the instrument.

Figure 4.2 Digital images of samples for density test via Ultra-pycnometer instrument

4.2.4 Specific capacity \( (C_p) \)

Temperature-modulated differential scanning calorimetry (TMDSC) measurements are performed using a TA Instruments Q 200, equipped with a refrigerated cooling
system. All samples are heated at 2°/min with a modulation period of 60 s and temperature amplitude of 0.318°. Sapphire reference standard is used for calibration of heat capacity. The heat capacity measurement consists of three runs. The first run is empty Al sample pan versus empty Al reference pan to obtain the cell asymmetry and baseline correction [206]. The second run is sapphire standard versus empty Al reference pan to calibrate heat flow amplitude according to standard equations [207, 208]. The third run is sample versus empty Al reference pan. The same empty Al reference pan is used in all the runs, and all the Al sample pans were kept the same in weight. Three tests are averaged for each sample to get the $C_p$ values.

4.2.5 Thermal diffusivity $D(T)$

Thermal diffusivity is measured with a transversal laser-flash apparatus (NETZSCH LFA 457 MicroFlash®). An energy pulse heats one side of a plane-parallel sample (See Figure 4.3). The thermal diffusivity $\alpha$ is calculated from the temperature rise on the backside as follows [209]:

$$\alpha = 0.1388 \times \frac{d^2}{t_{1/2}} \quad (\text{Equation 4-1})$$

where $\alpha$ is the thermal diffusivity; $d$ is the thickness of the sample; $t_{1/2}$ is the time to the half maximum of temperature at the backside (See Figure 4.3).
Figure 4.3 Schematic of LFA 457 measurement and sample preparation

Thermal conductivities $\lambda (T)$ perpendicular to samples are calculated from heat diffusivity $\alpha (T)$, specific heat capacity $C_p (T)$, and the mass density $\rho (T)$ using the relation $\lambda (T) = \alpha (T) \rho(T) C_p (T)$ with the built-in analysis software. As the variation of $\rho$ with $T$ is well within the accuracy of the thermal measurements, room temperature values were employed: $\rho (B. mori) = 1.347 \, \text{g/cm}^3$; $\rho (A. pernyi outermost) = 1.371 \, \text{g/cm}^3$; $\rho (A. pernyi innermost) = 1.365 \, \text{g/cm}^3$. Finally, the phenomenological heat transport equation, $\lambda (T) = C_{ph,v} \theta l_{ph}/3$ ($\theta$ is the sound velocity; $l_{ph}$ is the MFP of phonons), enables $l_{ph}$ to be calculated in order to compare it with the crystallite size [150].

4.2.6 Characterisation of the longitudinal section and cross sectional surfaces of a single fibre

Silk fibres are embedded in epoxy resin and cut with a LBK Ultramicrotome to get thin slices ($\sim 500 \, \text{nm}$), respectively. The longitudinal slice is coated with gold and analysed with a scanning electron microscope (SEM) (Zeiss Supra 55VP) at an accelerating voltage of 5 kV and a working distance of 8 mm. The transverse slice is
scanned by an atomic force microscope (AFM) (Bruker MultiMode™ 8 SPM instrument) in ScanAsyst mode with a silicon tip. The scanning images are based on a 5 μm × 5 μm region with a scan rate of 0.977 Hz.

4.3 Results and discussion

4.3.1 Thermal conductivity of cocoon layers, sericin and fibroin fibres

Silkworm cocoons are important biological materials that protect silkworms from environment threat and predator attacks. Both the wild *A. pernyi* silkworm cocoon and the domestic *B. mori* silkworm cocoon have several layers. To understand the thermal properties of cocoon layers, the temperature profiles of outer and inner layers from *A. pernyi* and *B. mori* are measured at temperatures of 25, 35, 50 and 65 °C, respectively. The results are shown in Figure 4.4.
As shown in Figure 4.4, the thermal diffusivities of the outer and inner layers from *B. mori* cocoon, which are about 0.4 and 0.3 mm$^2$/s, respectively, do not show much
difference. The thermal conductivities of the two layers, though slightly increasing along with temperature, have very similar values at around of 0.15 W/m·K, which is far below those of the *A. pernyi* cocoon layers. The small temperature lag between outer and inner layers of *B. mori* will facilitate the thermal transfer when the surrounding conditions are changed. Despite of more sericin content in the outer layer, the low thermal diffusivity of this layer should be partially due to the fact that more still air is trapped within this layer as a result of low fibre density [210]. This makes heat transfer much more slowly to reach the other side. The thermal conductivity of the outer layer of *B. mori* cocoon, however, is slightly higher than that of the inner layer, indicating the outer layer has a larger value of specific capacity. The results indicate that even though *B. mori* cocoon layers do not show much difference, the outer layer is more helpful to regulate the temperature inside the cocoon.

In contrast, the *A. pernyi* cocoon layers exhibited relatively greater thermal buffering capacity over *B. mori* cocoons. The outer layer of *A. pernyi* shows much higher thermal diffusivity/conductivity than that of inner layer, demonstrating a significant thermal damping characteristic against temperature fluctuations. As illustrated in chapter 2, the outer layer has a lower fibre density than the inner layer, which is not beneficial to heat conduction. Its superior thermal diffusivity/conductivity is probably attributable to the presence of a large amount of calcium oxalate crystals and higher sericin content.

To understand the high thermal diffusivity/conductivity of the outer layer of the *A. pernyi* cocoon, the thermal properties of the crystals and sericin as well as fibroin fibres, are shown in Figure 4.5. As the thermal diffusivity/conductivity values of the outer and inner layers of *B. mori* do not show much difference, the degummed silk fibres from *B. mori* cocoon as a whole were tested.
Figure 4.5 The thermal diffusivity/conductivity of degummed silk fibres and sericin as well as calcium oxalate crystals versus temperature. (a) and (b) *A. pernyi*; (c) and (d) *B. mori*.

A comparison between Figure 4.5 and Figure 4.4 shows that, the thermal diffusivity/conductivity of degummed silk fibre pellet is almost one order of magnitude smaller than the raw silk layers. Pellets of degummed *A. pernyi* silk fibres have a slightly higher diffusivity/conductivity (α: 0.03~0.05 mm²/s; λ: 0.04~0.05 W/mK), compared to the pellet of degummed *B. mori* fibres (α: 0.01~0.02 mm²/s; λ: 0.02~0.025 W/mK).

As expected, calcium oxalate crystals contribute significantly to thermal diffusivity/conductivity, followed by silk sericin. Although the diffusivity and conductivity of calcium oxalate crystals are obviously much lower than those of raw
cocoon layers, they are almost 9 and 13 times, respectively, that of degummed *A. pernyi* silk fibre pellet. In addition, the thermal diffusivity and conductivity of *A. pernyi* sericin (\(\alpha: 0.145 \text{ mm}^2/\text{s}; \lambda: \sim 0.35 \text{ W/m-K}) are higher than those of *B. mori* sericin (\(\alpha: 0.135 \text{ mm}^2/\text{s}; \lambda: \sim 0.27 \text{ W/m-K}).

The thermal diffusivity of degummed silk fibre is obtained along its transverse direction. As the fibre pellet is pressed under 10 tons, the negative effect of still air and the thermal resistance between fibres can be neglected. The thermal diffusivity/conductivity of a single fibre could be equal to that of a bundle of fibres [151]. Therefore, in this study, except for the raw silk layers, the thermal diffusivity/conductivity values of fibre pellets and fibre bundles are assumed to be equal to that of a single fibre in transverse and longitudinal direction, respectively.

4.3.2 Thermal conductivity/diffusivity of degummed silk fibres in longitudinal direction

Since the long peptide chains are aligned along the fibre axis, it is expected that the thermal diffusivity/conductivity should be higher along the longitudinal direction. The thermal diffusivities of *A. pernyi* and *B. mori* silk fibres along this direction are shown in Figure 4.6.
Figure 4.6 The thermal diffusivity/conductivity of degummed *A. pernyi* and *B. mori* silk fibres in longitudinal direction

Similar to their thermal transfer in the transverse direction, the thermal diffusivity/conductivity of inner fibres in the longitudinal direction is obviously higher than that of outer fibres. As illustrated in chapter 3, compared to fibres from the outer
cocoon layer, fibres from inner layer of the *A. pernyi* cocoon have relatively stable and higher β-sheet content, which may contribute to their better thermal conduction. It is worth mentioning that, the thermal diffusivity/conductivity in longitudinal direction is one order of magnitude larger than that in transverse direction. This difference indicates anisotropic thermal transfer in the two directions. The highly oriented alanine-rich nanocrystals of antiparallel β-pleated sheets along the fibre axis may play a significant role in the heat transfer along fibre axis. In contrast, the heat transfer in transverse direction is, mostly phonon-scattered due to crystal-crystal barriers and therefore, thermal diffusivity in this direction is lower. This anisotropic behaviour is more obvious for *B. mori* silk fibres. Despite of lower thermal transfer in transverse direction, *B. mori* fibres have an extremely high thermal diffusivity/conductivity in longitudinal direction. This is closely related to its intrinsic structure. The high orientation and crystallinity bestow *B. mori* silk fibres superior thermal transportation in this direction.

Other natural fibres, like cotton, wool and ramie, are also tested to get their thermal properties and the results are shown in Figure 4.7. Among these natural fibres, silk and wool have close values of $\alpha (T)$ and $\lambda (T)$ in the range of 0.2–0.4 W/m·K and 0.3–0.7 mm$^2$/s, respectively. Wool fibres have relatively low values, with a small fluctuation at about 0.2 mm$^2$/s for $\alpha (T)$ and 0.5 W/m·K for $\lambda (T)$. In contrast, the $\alpha (T)$ and $\lambda (T)$ of cotton and ramie fibres are twice those of silk and wool fibres, probably due to their high crystallinities of 81% and 85%, respectively [211]. As discussed in chapter 1, apart from the intrinsic structure of a fibre, moisture adsorption is an important factor that affects its thermal properties [212, 213]. Cotton is characteristic of strong moisture adsorption. The moisture would increase specific heat and density and therefore, a great increase in thermal conductivity of cotton is shown in Figure
4.7 (b) In addition, the unique lumen (hollow microtube) inside ramie fibre probably has a negative effect on its thermal conductivity [214].

Figure 4.7 Thermal diffusivities along longitudinal direction of cotton, wool and ramie
4.3.3 The crystallite size versus thermal transportation

To identify the main factor for thermal conductive properties, the thermal diffusivity in the longitudinal direction as a function of the β-sheet contents and the crystallite size are shown in Figure 4.8.

![Figure 4.8](image)

**Figure 4.8** The effects of β-sheets on thermal diffusivity (a) and the crystallite size effect on the thermal diffusivity of (b) *A. pernyi* outermost fibres; (c) *A. pernyi* innermost fibres; (d) *B. mori* silk fibres.

β-sheets include intramolecular β-sheets and intermolecular β-sheets. As shown in Figure 4.8 (a), $\alpha(T)$ of *A. pernyi* outermost fibre (0.2~0.42 mm²/s) increases obviously along with the increase of intramolecular β-sheets (5~27%). However, $\alpha$ of *A. pernyi* innermost fibres (0.33~0.34 mm²/s) increases only slightly with the increase of intramolecular β-sheets (3.83~15.09%), and then decreases with a further increase in
intramolecular β-sheets (15.09~25.11%). The same trend is observed for α of B. mori silk fibres with the increase of intramolecular β-sheets (3.48~36.72%, 0.383~0.473 mm²/s). The intramolecular β-sheets have polypeptide chain bent onto itself and one peptide is connected with its counterpart via an intramolecular hydrogen bonding. When heat flows along the peptide, the intramolecular hydrogen bonding may act as a fast channel for heat to pass through. The highest values of α are achieved when the intramolecular β-sheets contents are 27% for A. pernyi outermost fibres, 15.23% for A. pernyi innermost fibres, and 25.63% for B. mori silk fibres, respectively. The intermolecular β-sheets constitute the crystalline parts of silk fibres and are aligned along the longitudinal direction of a silk fibre. Therefore, the size of crystallites may affect the thermal conduction in the longitudinal direction. As shown in Figure 4.8 (b,c,d), an increase in crystallite size promotes the thermal conduction of the A. pernyi outermost fibres. The highest value of α is achieved when the crystallite size increases to 4.17 nm. In contrast, A. pernyi innermost fibres and B. mori silk fibres exhibit slight increase in α and maximal α are obtained when the crystallite size reaches 4.26 and 2.82 nm, respectively, followed by decrease with a further increase in crystallite size. This means bigger crystallite size of A. pernyi innermost and B. mori silk fibres does not necessarily lead to better thermal conduction. For all the fibres, temperature has a negative effect on their thermal diffusivity.

The corresponding orthorhombic lattice constants, a, b, c at the optimal crystallite sizes, which are 4.17, 4.26 and 2.18 nm, for the A. pernyi outermost fibres, A. pernyi innermost fibres and B. mori silk fibres have been given in Table 3.1. Combining this table and Figure 4.8, it can be concluded that, increasing β-sheet stacking (a axis) and the β-strand direction (c axis) can increase the thermal conduction of A. pernyi outermost fibres, whereas increasing β-sheet stacking (a axis) and the number of
chains ($b$ axis) are intended to increase thermal conduction of *A. pernyi* innermost fibre and *B. mori* silk fibres.

4.3.4 Phonon mean free path (MFP) in nanocrystals

Structurally, the $\beta$-sheet structure of silk consists of aligned polypeptide strands interlocked by hydrogen bonds, which can blue-shift the frequencies of the phonon modes and further enhance thermal conduction. To illustrate the thermal transport process in $\beta$-sheets, the influence of intermolecular $\beta$-sheet only is analysed in this study, due to its dominant role in thermal conduction of *A. pernyi* silk. Figure 4.9 illustrates the crystallite structure of intermolecular $\beta$-sheets of *A. pernyi* silk.
Figure 4.9 Crystallite structure. (a) β-sheet crystallite structure in silk fibre; (b) the three dimensional diagram of the enlarged β-sheet crystallite in (a); (c) Side view of the β-sheet crystallite consisting of 5 single peptide chains; (d) Structure of a four-layer β-sheet crystallite. Models of *A. pernyi* anti-parallel β crystals were constructed using Discovery Studio software with unit cell parameters from X-ray diffractions.

Because the total thermal conductivity at low temperatures is very small, the phonon contribution would be significant. For electronically insulating materials, heat transport is mainly by means of acoustic phonons. According to the phenomenological heat transport equation, \( k = \rho C_p \vartheta l_{ph}/3 \) or \( \alpha = \vartheta l_{ph}/3 \), where \( \vartheta \) is sound speed, the phonon MFP \( l_{ph} \) could be calculated. Here the case at 25 °C is used as an example to
estimate the upper limit of the lattice thermal conductivity. The acoustic phonon group velocity is conventionally dominated by the covalent bonds (i.e. C-C, C-O) [136]. The average sound velocity can be directly converted from Young’s modulus via the relationship \( v = (E/\rho)^{0.5} \) [215, 216]. According to Figure 3.8 (c) and the density of these fibres, the sound velocities are calculated to be 3663 and 3428 m/s for innermost and outermost fibres. Therefore, the MFP of phonon could be calculated as 0.17 nm and 0.29 nm for outer fibre and inner fibre, respectively. As calculated in chapter 3, the crystallite sizes of outer fibre and inner fibre are 3.58 and 3.54 nm, respectively, which are far longer than their phonon MFP. This means minimal phonon scattering occurs at interfaces of crystals and hence the nanocrystals may have excellent thermal conduction [217]. If the phonon MFP exceeds the lateral dimensions of single crystals, the phonon scattering at interfaces can significantly reduce thermal conductivity [136]. On the other hand, for single crystalline materials that are defect-free, a bigger MFP will result in higher thermal conduction [218]. Compared to outermost fibres, innermost fibres have higher phonon MFP, which reduces interfacial scattering of the phonons and increase thermal conductivity.

4.3.5 Porosity

Except MFP, porosity is another dominant factor to reduce thermal conductivity in nanocrystalline materials [142]. As studied in chapter 2, A. pernyi silk fibres are porous, which is markedly different from B. mori fibres. Figure 4.10 shows the pores of A. pernyi silk fibres with B. mori silk fibres as comparison.
**Figure 4.10** The geometry of pores in *A. pernyi* and the cross section of *B. mori* silk fibres. (a) The longitudinal section of a single *A. pernyi* fibre; (b) The enlarged view of (a); (c) The cross-sectional area of a single *A. pernyi* fibre; Inset is the numerical depth values in (c) across the sections indicated by the solid line; (d) The solid cross sectional area of *B. mori* silk fibres.

**Figure 4.10** (a) and (b) shows the pores in the longitudinal direction of silk fibre and its size ranges from 50 nm up to 400 nm. **Figure 4.10** (c) is an images of the cross section of an *A. pernyi* fibre, which show that the pores are isolated from each other to form closed cells. This can also be clearly seen from the depth mapping (Insert of **Figure 4.10** (c)), which indicates the depth of the holes are less than 20 nm. Since enclosed cells are probably filled with still air, its thermal conductivity/diffusivity can be equal to that of still air, *i.e.* 0.023W/m·K and 0.079 mm/s, respectively.
It is obvious from Figure 4.10 (b) that the pore size of *A. pernyi* fibres ranges from 100 to almost 600 nm, which is far bigger than that of the crystallites in the fibres. The crystallite is not totally aligned along the fibre axis, which can be inferred from its greatly increased strength after stretching [9]. However, an order of magnitude of pore size may tilt a crystallite to a certain angle along the pores. Therefore, the effective thermal conductive properties of crystallites are mainly determined by the cosine values that form certain values with it. Taking the angle of the β-sheet as θ and neglecting the thermal transportation between chains of β-sheet, the effective thermal conduction in the longitudinal direction of the silk fibre, \( k_{\text{eff}} \) equals to \( k \cos^2 \theta \) (Figure 4.11), where \( k \) is the average thermal conductivity in the chain direction of β-sheet block. Therefore, when \( \theta \) is 90 degree, the average thermal conductivity in the chain direction will not contribute to the total thermal conduction along the fibre axis. Instead the thermal transfer among different chains will contribute to the overall thermal conductivity for this crystal.

![Figure 4.11](image)

**Figure 4.11** (a) The model of the silkworm structure; (b) the various degree of inclination of β-sheet blocks
In a single silk fibre, crystallites are embedded in non-crystalline structures. Therefore, a fibre composite has a heterogeneous structure consisting of crystallites, intramolecular β-sheets, amorphous region and pores. The presence of multiple components increases the phonon scattering, affecting the thermal conductivity of a whole fibre. As discussed above, these components exhibit various structures varying thermal properties. This is evident from the thermal diffusivity/conductivity of different silk fibres from *A. pernyi*, i.e. outermost and innermost fibres. Therefore, although the thermal conduction of this semi-crystalline composite is complex, a fibre with tailored thermal property can be fabricated by the proper choice of constituents and configuration.

4.3.6 Secondary structure versus thermal diffusivity/conductivity

It is striking that the great anisotropy of thermal conduction in transverse and longitudinal directions can lead to such distinct thermal conducting capabilities. The main contributor to the differences in thermal conduction is the hydrogen bonding between the β-strands (i.e. the polypeptide strands) that form the β-sheets along the fibre axis.

To further illustrate the effect of the density of hydrogen bonding on its thermal property, the regenerated *A. pernyi* fibroin films with a gradient of crystallinity were measured for their thermal properties.
Figure 4.12 XRD curves of *A. pernyi* silk fibroin films induced by 80% methanol (a) with a gradient of crystallinity (b) and their thermal diffusivities (c) and conductivities (d).

The regenerated fibroin film is characteristic of an amorphous structure. As indicated in Figure 4.12, these curves show two broad peaks at around 12.2 and 23.5° and a
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shoulder peak at about 28°, which are known to be reflections of helices components of A. pernyi fibroin [36, 219, 220]. These curves, however, are apparently overlapped with halos from amorphous components, indicating the presence of significant amounts of random coil. The regenerated fibroin film with significant amounts of amorphous phase has a crystallinity of only about 15%. The thermal diffusivity and conductivity in this state are only about 0.02 mm²/s and 0.03 W/m·K, respectively. By immersing in methanol solution, the rearrangement of the inter/intra-molecular hydrogen bonds would have occurred, and this should cause a conformational change from the amorphous structure to the β-sheet structure. The hydrogen-bonded crystalline protein films have a β-sheet content with an abrupt increase to 40% and 51% after being treated in methanol for 5 and 60 min, respectively. The thermal diffusivities/conductivities of the treated films are almost 6 times higher than those of original/untreated film (See Figure 4.12 (c) and (d)). The increase rate of thermal conductivity along with temperatures indicates that, with the increase of β-sheet, thermal conductivity increases obviously as a function of temperature. This emphases the positive contributions of hydrogen bonds in facilitating thermal conduction in β-sheet. This analysis indicates that by changing the density of hydrogen bonds, the thermal property can be tuned.

4.4 Conclusions

Silkworm cocoons are able to provide significant buffer against temperature changes outside of the cocoon structure. The A. pernyi cocoon has a stronger thermal buffer function over the B. mori cocoon. The degummed A. pernyi silk fibres also have an obviously higher thermal conduction than that of B. mori in transverse direction. Both silk and wool fibres have similar thermal conduction in longitudinal direction, but their
thermal conductivity is much lower than that of ramie and cotton fibres. The size effect of the nanocrystallites in longitudinal direction on the thermal conduction indicates, the superior $\alpha$ values are exhibited at the optimal crystallite size at about 4.17, 4.26 and 2.82 nm for *A. pernyi* outermost, innermost and *B. mori* silk fibres, respectively. Increasing $\beta$-sheet stacking ($a$ axis) and the $\beta$-strand direction ($c$ axis) have the tendency to promote the thermal conduction of outermost fibres, whereas increasing $\beta$-sheet stacking ($a$ axis) and the number of chains ($b$ axis) are intended to increase thermal conduction of *A. pernyi* innermost fibre and *B. mori* silk fibres. In spite of the same polypeptide sequence in outermost and innermost fibre from *A. pernyi*, the large phonon MFP in inner fibre makes its $\lambda(T)$ and $\alpha(T)$ much higher than those of outer fibre. Changing crystallinity degree has been proven to be effective for tuning the thermal property of silk fibres, and the $\lambda(T)$ and $\alpha(T)$ with high crystallinity are more sensitive to temperature. The characteristic pores in *A. pernyi* silk fibre greatly reduce the heat transfer due to the inclination degree of the nano crystallite along the relatively large nano pores. The results may further guide the design of synthetic silk materials to achieve tuneable thermal properties.
CHAPTER 5  Interactions between fibroin and sericin proteins from *A. pernyi* and *B. mori* silk fibres

5.1 Introduction

As explained in chapter 2, the morphology and the structure of *A. pernyi* silk fibres decide its mechanical properties and protective functions. A silkworm silk fibre is a typical natural (core-shell) composite, consisting of two fibroin core fibres glued with sericin coatings. Sericin coating, as a protective layer, has an excellent impact resistance and aging resistance [221]. Compared to *B. mori*, under the same conditions (temperature, salt concentration and treatment duration, etc), degumming *A. pernyi* fibres is more difficult, although they have a lower sericin content. Sericin can be easily removed from *B. mori* silk by alkaline (sodium carbonate) or hot water (Figure 5.1(a) and (b)). However, they can only partially remove the sericin from the *A. pernyi* silk under the same conditions (Figure 5.1(c) and (d)). Therefore, stronger interactions must exist between fibroin and sericin of *A. pernyi*. The stronger interactions could contribute to the excellent properties of this wild silkworm cocoons, protecting the worm from attack by predators or abrasion against twigs.
In fact, inspired by nature, many types of composite materials with super mechanical properties and functions have been designed. For example, PEE/PPy composite films inspired by the network structure of animal dermis [222], artificial composite with high resilience inspired by nacre [223], and superhydrophobic surface inspired by lotus leaf [224], have been developed for different applications.

So far, a fundamental understanding of the adhesion between sericin and fibroin of the wild silk has not been achieved. The knowledge could inspire the design of composite materials with excellent interfacial adhesion between the different components and superior properties. In this chapter, the interactions between sericin and fibroin are systematically analysed via Inverse Gas Chromatography (IGC), Fourier Transform Infrared Spectroscopy (FTIR), Proton Nuclear Magnetic Resonance (NMR), Quartz
Crystal Microbalance with Dissipation (QCM-D) and Contact Angle (CA). This study will help develop suitable silk processing (e.g. degumming) methods and inspire new biomimetic composite materials.

5.2 Experimental

5.2.1 Native silk cocoons

*A. pernyi* silkworm cocoons were collected from North China and *B. mori* cocoons were purchased from the silk rearing house in China.

5.2.2 Reagents

The details on reagents for silk degumming, fibre dissolving, desalting, have been given in chapter 4, section 4.2.2.

Deuteroxide (D2O, 99%, Sigma-Aldrich) was used for NMR tests.

5.2.3 Methods

1) Preparation of sericin proteins

The details on silk degumming have been given in chapter 4, section 4.2.1.

2) Preparation of fibroin proteins

Degummed *A. pernyi* fibroin fibres are dissolved in a LiSCN (10 M) at 55 °C for 2 h with a fibre mass (g) to solvent volume (mL) ratio of 1:10. After filtration, the solution is dialyzed in a cellulose tube against DI water at 4 °C for 4 days. The solution is then centrifuged to remove aggregates. The fibroin solution is then freeze dried to obtain fibroin protein.
Degummed *B. mori* fibroin fibres are dissolved in a ternary solvent made of CaCl₂, H₂O, C₂H₅OH solution at a molar ratio of 1:8:2 with a fibre mass (g) to liquor volume (mL) ratio of 1:10 at 75 °C for 2 h [158]. The solution is then filtrated, dialyzed for 4 days before being freeze-dried.

(3) Characterization of degummed silk fibres

The degummed silk fibres are analysed with a scanning electron microscope (SEM) (Zeiss Supra 55VP) at an accelerating voltage of 5 kV and a working distance of 8 mm. The fibres are coated with a thin layer of gold using a sputter coater (LEICA EM ACE600).

Surface roughness of the degummed silk fibres is analysed with a scanning probe microscope (SPM) (Bruker MultiMode™ 8) in ScanAsyst mode with a silicon tip. The degummed silk fibres are mounted onto silicon wafers. The scanning images are based on a 5 μm × 5 μm region with a scan rate of 0.977 Hz. The surface roughness is expressed as Ra, which is the arithmetic average of the absolute values of the surface height deviations.

(4) Work of adhesion between sericin and degummed silk fibres

An IGC – Surface Energy Analyzer (Surface Measurement System, Alperton, Middlesex, UK) is used to determine the work of adhesion between sericin and degummed silk fibres. 1.5 g of degummed fibres and 60 mg of sericin powders are packed into a salinized glass columns that are 300 mm in length and 4 mm in inner diameter.

The surface areas are measured and determined using octane sorption isotherms while the profiles and the distributions of the surface energy components are determined directly using a combination of dispersive and polar probe molecules, including n-
heptane, n-octane, n-hexane, n-nonane, chloroform, ethyl acetate, acetone, dichloromethane and 1,4-dioxane. The molecular concentrations of the probes are converted to a fractional surface coverage on the basis of their molecular cross-sectional area. The surface coverage is fitted to an exponential function of surface energy including dispersive surface energy ($\gamma_S^D$) and specific surface energy ($\gamma_S^{AB}$), respectively. $\gamma_S^D$ relates to non-specific interactions via physical process based on the retention of a series of injected n-alkane adsorbates (n-heptane, n-octane, n-hexane and n-nonane). $\gamma_S^{AB}$ relates to interactions occurring via chemism such as hydrogen bonding and acid-base forces by using polar probes (chloroform, ethyl acetate, acetone, dichloromethane and 1,4-dioxane) [95].

The work of adhesion between fibroin and sericin is determined with ethyl acetate and chloroform as reference substances. The retention volumes are obtained from the Peak Com method. These Peak Com measurements are undertaken at surface coverages of 1.5%, 2%, 4%, 6%, 8%, 10%, 20% and 35%, respectively. Calculations were performed using the SMS Analysis Software v1.2. The data are fit with an exponential decay function $y = y_0 + Ae^{-x/t}$. 

(5) Adsorption of sericin on silk fibroin films

QCM-D (Q-SENSE, Västra Frölunda, Sweden) is used to characterize the adsorption of sericins on fibroin films. The sensor crystals used are 5 MHz, AT-cut, polished quartz discs (chips) with gold electrodes. Silk fibroin (1 mg/mL) is deposited onto the gold crystal followed by rinsing with DI water. Due to the hydrophobic interaction, the adsorbed fibroin film is very stable as no drift in the baseline frequency is observed during rinsing with DI water. Sericin (1 mg/mL) is then filtered through a 0.45 μm membrane syringe filter and dropped onto the fibroin film. After the adsorption of
sericin reached an equilibrium, the film is rinsed with DI water. The adsorbed sericin can be deducted from the Sauerbery equation. The changes in resonance frequency ($\Delta f$) and energy dissipation ($\Delta D$) are measured simultaneously at the 5th and 7th harmonics. All tests are carried out in an air-conditioned room at 20 °C. The raw data is analysed using QTools (Q-SENSE) and Origin 9 (Origin Lab, USA) software.

(6) Molecular interactions between sericin and fibroin

$^1$H NMR and FTIR are used to probe the interactions between sericin and fibroin. For $^1$H NMR study, silk fibroin and sericin proteins from A. pernyi and B. mori fibres are dissolved in D$_2$O at a concentration of 1 wt%. Fibroin and sericin solutions are then mixed at a volume ratio of 1:1. $^1$H NMR spectra and diffusion coefficients are recorded on a Bruker Advance III 500 MHz wide-bore spectrometer (1H Larmor frequency of 500.07 MHz) equipped with a 5 mm Diff50 pulse-field gradient (PFG) probe. PFG-stimulated echo pulse sequence is used to measure the diffusion coefficients. The maximum gradient amplifier strength is 29.454 T/m. The diffusion coefficients are calculated from the following equation:

$$\ln(I/I_0) = -D_{\text{NMR}} \gamma^2 (\Delta - \frac{\delta}{3}) \delta^2 g^2$$  \hspace{1cm} (Equation 5-1)

where $I$ and $I_0$ are the NMR signals with and without the presence of PFG, respectively; $\gamma$ is the gyromagnetic ratio of proton; $\delta$ is the gradient pulse length, $\Delta$ is the interval between the gradient pulses; $g$ is gradient strength. In this study, $\Delta$ and $\delta$ is set to 100 ms and 2 ms, respectively. The gradient strength is optimized according to the diffusion coefficient. 16 gradient steps and 32 scans for each step are used for all the measurements.

For FTIR analysis, equal volumes of fibroin and sericin, both at a concentration at 1 mg/mL, are mixed, and followed by freeze drying. The lyophilized powders would
provide enough interfacial area between sericin and fibroin and also avoid interference from the IR absorption of water vapour. Attenuated Total Reflectance (FTIR – ATR) spectra of lyophilized powders are recorded (4000 – 600 cm\(^{-1}\)) on a Bruker Vertex 70 FTIR spectrometer. All spectra are averaged from 64 scans at a resolution of 4 cm\(^{-1}\).

(7) Contact angle

Contact angle (CA) measurements are conducted with a Malvern contact angle measurement instrument (KSV CAM101 Instrument Ltd) equipped with a CCD camera using 13 µL fluid droplets. The dynamic contact angle as a function of the post-reaction time in the dry state is conducted by dropping sericin protein at a concentration of 1 mg/mL onto fibroin films. Time is set to zero immediately after the drop is deposited on the surface, at this time the first contact angle is measured. To confirm the uniform distribution of fibroin film, the contact angle is measured three times from different positions and an average value is calculated by statistical method. As a control experiment, the water contact angle is also done to phase out the effect of water in sericin solution.

5.3 Results and discussion

5.3.1 Work of adhesion between sericin and fibroin by IGC

The work of adhesion is defined as the work required to build a unit area of a particular surface. Thus, surface energy is related to the work required to cleave a bulk sample, creating two sub-surfaces. A higher work of adhesion suggests stronger interactions. **Figure 5.2** shows the work of adhesion profiles between fibroin and sericin as a function of surface coverage of degummed fibres. The total work of adhesion (\(W_s\))
demonstrates that fibroin and sericin from *A. pernyi* (AF&AS) has a stronger interaction than that between fibroin and sericin from *B. mori*. (BF&BS).

Figure 5.2 Work of adhesion profiles between fibroin and sericin as a function of fractional surface coverage of silk materials. BF and BS denote *B. mori* fibroin and sericin, respectively; AF and AS denote fibroin and sericin from *A. pernyi*, respectively.

The surface energy distribution via IGC is pressure-dependent, appropriate for the determination of heterogeneity profiles and sensitive to small differences between energetic levels. The work of adhesion (or binding energy) can be determined by the surface energy of a material. A flat line of work of adhesion versus coverage of a surface with an adsorbate is indicative of a homogeneous adhesion surface. The total work of adhesion between sericin and fibroin decreases with an increase in surface coverage and stabilizes when the surface coverage reaches a certain/critical value, which is 0.12 and 0.16 for *B. mori* and *A. pernyi*, respectively. The decrease in work
of adhesion indicates heterogeneity of interactions between fibroin and sericin and a higher critical surface coverage value indicates a higher degree of heterogeneity. Therefore, the interaction between sericin and fibroin from *A. pernyi* fibres is more heterogeneous.

The total work of adhesion ($W_S^{T}$) includes contributions from both dispersive (non-specific) interactions ($W_S^{rd}$) and specific interactions ($W_S^{sb}$) [225]. Similar to the total work of adhesion, both the dispersive and specific work of adhesion decrease with an increase in surface coverage and approach constant values, indicating heterogeneity in both types of adhesion. However, it is noteworthy that in contrast to the specific interactions, the dispersive work of adhesion between fibroin and sericin from *A. pernyi* is only slightly higher than the value between fibroin and sericin from *B. mori*.

Table 5.1 gives the average (constant), maximum (at zero surface coverage) and range (maximum value-average value) of different types of work of adhesion, as well as their decay constants. The larger decay constant and the larger difference between the maximal and average work of adhesion also indicate the adhesion between sericin and fibroin from *A. pernyi* fibres is more heterogeneous.

<table>
<thead>
<tr>
<th>Work of Adhesion</th>
<th>Sample</th>
<th>Adhesion</th>
<th>Decay constant (t)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average y(1)$^a$</td>
<td>Maximum (A)</td>
<td>Range (A)</td>
</tr>
<tr>
<td>Total</td>
<td>BF&amp;BS</td>
<td>140</td>
<td>246</td>
</tr>
<tr>
<td></td>
<td>AF&amp;AS</td>
<td>152</td>
<td>313</td>
</tr>
<tr>
<td>Dispersive</td>
<td>BF&amp;BS</td>
<td>94</td>
<td>144</td>
</tr>
<tr>
<td></td>
<td>AF&amp;AS</td>
<td>97</td>
<td>171</td>
</tr>
<tr>
<td>Specific</td>
<td>BF&amp;BS</td>
<td>47</td>
<td>107</td>
</tr>
<tr>
<td></td>
<td>AF&amp;AS</td>
<td>55</td>
<td>142</td>
</tr>
</tbody>
</table>
a) $y(1)$ is the average surface energy at the stabilization stage, which is equal to the value when surface coverage is 100%; maximum denotes the maximal work of adhesion at surface coverage of 0%, calculated from the exponential equation; the decay constant gives an indication of the heterogeneity.

The dispersive interactions arise from long-range London dispersion forces (van der Waals force), which is mainly influenced by the surface topographies. A higher specific surface area (surface area per unit mass) means a higher capacity for adsorption. SPM characterization shows ordered alignment of nanofibrils in silk fibres, and the roughness result indicates that the surface of *A. pernyi* silk fibrils is pretty bumpy (Figure 5.3). The average roughness of fibres from *A. pernyi* cocoon and *B. mori* cocoon is 12.6 nm and 4.8 nm, respectively. The higher roughness of *A. pernyi* fibres is conducive to adsorption of sericin [4], which explains the slightly higher values of dispersive work of adhesion between fibroin and sericin from these fibres.

![Figure 5.3](image)

**Figure 5.3** Roughness maps of the silk fibrils based on a 5 um × 5 um region. (a) *A. pernyi* cocoon fiber; (b) *B. mori* cocoon fiber
Chapter 5 Adhesion between fibroin and sericin

The specific adhesion is due to short-range chemical interactions which include charge redistribution, such as hydrogen bonding [226]. As shown in Figure 4.2 and Table 4.1, in contrast to the dispersive adhesion, the specific adhesion between fibroin and sericin from *A. pernyi* (about 56 kJ/mol) was significantly higher than that from *B. mori* (about 46 kJ/mol), indicating *A. pernyi* fibroin fibres have more active surface for the adsorption of sericin.

5.3.2 Adsorption of sericin on fibroin films by QCM-D

To investigate the dynamic adsorption of sericin on fibroin, QCM-D was used to monitor the adsorption process. The QCM technique is extremely sensitive for mass adsorption at trace levels. It is based on the tendency of a piezoelectric crystal to change its natural oscillation frequency with mass deposition or depletion on it. According to Sauerbrey [227] and Rodahl [228], oscillation frequency of the crystal can be calculated from equations (5-2) and (5-3).

\[
\Delta f = -\frac{2F_0 n}{\sqrt{A} t_q \rho_q} \Delta m = -C \Delta m \quad \text{(Equation 5-2)}
\]

\[
D = \frac{E_{\text{dissipated}}}{2\pi E_{\text{stored}}} \quad \text{(Equation 5-3)}
\]

where \(\Delta f\), \(n\), \(F_0\), \(A\), \(t_q\) and \(\rho_q\) are the frequency change in Hz, overtone number, fundamental frequency of the quartz sensor, the surface area of the piezoelectric region of the sensor, the sensor thickness, and the sensor density respectively. \(\Delta m\) (ng/cm\(^2\)) is mass change per unit area. For this system, \(C\) is the sensitivity factor of the crystal in Hz/ng/cm\(^2\) (17.7 ng/cm\(^2\)/Hz for a 5 MHz at 20 °C). \(E_{\text{dissipated}}\) is the energy dissipation during one oscillation cycle and \(E_{\text{stored}}\) is the total energy stored in the oscillating crystal.
Figure 5.4 Adsorption of sericin on fibroin films as studied by QCM. (a) The mass change calculated from Sauerbrey equation as a function of deposition time; (b) QCM in-situ frequency change as a function of deposition time; (c) $\Delta D/\Delta f$ plot for the adsorption of sericin onto fibroin surfaces.
A representative in-situ frequency, dissipation and mass changes (function of time) for the adsorption of silk sericin on silk fibroin film surface is shown in Figure 5.4. The kinetics of sericin adsorption on fibroin surface typically consists of a very rapid initial adsorption phase, followed by a slower phase before approaching an equilibrium (Figure 5.4a). After rinsing with DI water, the amount of adsorbed B. mori sericin was significantly less than that of A. pernyi sericin. The A. pernyi sericin adsorbed (92 ng/cm²) is about twice of that of B. mori sericin (48 ng/cm²), which is indicative of more irreversible adsorption of A. pernyi sericin on A. pernyi fibroin. The results support the higher specific work of adhesion between proteins from A. pernyi. The higher affinity of A. pernyi sericin to fibroin is also indicated by the larger decrease of the resonance frequency of the crystal sensor (Figure 5.4b). As seen in Figure 4.4c, A. pernyi and B. mori display nearly the same adsorption behavior, with the ratio of $\Delta D/\Delta f$ of $0.7 \times 10^{-7}$ for both silks, indicating the adsorbed sericin films are rigid [229].

![Figure 5.5 Silk sericin immobilization onto fibroin film](image)
Figure 5.5 shows a monolayer adsorption on both fibroin films is formed, due to a single down trend of the frequency change [230], therefore, Langmuir isothermal adsorption model was introduced to describe the adsorption kinetics (Equation 5-4 and 5-5). Binding rate and Gibbs free energy were obtained from the following equations [231]:

\[ \Delta f = \Delta f_{\text{max}} \frac{k_a C}{k_a C + k_d} \left( 1 - e^{-\left( k_a C + k_d \right)t} \right) \]  
\[ K_{\text{equ}} = \frac{k_a}{k_d} \]  
\[ \Delta G = -RT \ln K_{\text{equ}} \]

(Equation 5-4)  
(Equation 5-5)  
(Equation 5-6)

where \( \Delta f_{\text{max}} \) is the maximum QCM resonance frequency shift; \( t \) is the time (s); \( C \) is the sericin concentration (M); \( k_a \) and \( k_d \) are adsorption and desorption rate constants, respectively. \( K_{\text{equ}} \) is equilibrium constant; \( \Delta G \) is free energy of adsorption (kJ/mol); \( R \) is the universal gas constant (0.008314 kJ/mol/K), \( T \) is temperature (in Kelvin).

Using Figure 5.4a and Equation (5-4), the average values of \( k_a \) and \( k_d \) for *A. pernyi* silk were determined to be 0.29 M/s and 1.33 \( \times 10^{-3} \) /s, respectively, and those for *B. mori* silk were found to be 0.17 M/s and 2.13 \( \times 10^{-3} \) /s. The equilibrium association constants, \( K_{\text{equ}} \), as calculated from Equation (5-5) are 216.05 M\(^{-1}\) and 81.70 M\(^{-1}\) for *A. pernyi* and *B. mori*, respectively (See Table 5.2). A higher value of \( K_{\text{equ}} \) indicates a higher affinity between molecules. The \( K_{\text{equ}} \) of *A. pernyi* silk is about 2.6 times of that of *B. mori* silk, meaning a higher specific affinity between sericin and fibroin from this wild silk. \( \Delta G \) is determined to be -13.10 kJ/mol for *A. pernyi* silk and -10.73 kJ/mol for *B. mori* silk, respectively. The lower \( \Delta G \) from *A. pernyi* also indicates a stronger affinity between its sericin and fibroin.
Table 5.2 The adsorption kinetic parameters determined using Langmuir model at 20°C

<table>
<thead>
<tr>
<th></th>
<th>$\Delta f_{max}$ (Hz)</th>
<th>Concentration of sericin (mM)</th>
<th>$k_a$ ($M^{-1}$s$^{-1}$)</th>
<th>$k_d$ ($\times 10^{-3}$ s$^{-1}$)</th>
<th>$K_{equ}$ (M$^{-1}$)</th>
<th>$\Delta G$ (kJ/M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. pernyi</td>
<td>5.36</td>
<td>18</td>
<td>0.298</td>
<td>1.9</td>
<td>156.842</td>
<td>-12.32</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.2869</td>
<td>1.1</td>
<td>260.818</td>
<td>-13.55</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.2796</td>
<td>1.0</td>
<td>279.6</td>
<td>-13.72</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
<td>0.288</td>
<td>1.333</td>
<td>216.054</td>
<td>-13.100</td>
</tr>
<tr>
<td>B. mori</td>
<td>2.69</td>
<td>25</td>
<td>0.162</td>
<td>2.5</td>
<td>64.8</td>
<td>-10.16</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.1914</td>
<td>1.9</td>
<td>100.737</td>
<td>-11.24</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.1694</td>
<td>2.0</td>
<td>84.7</td>
<td>-10.814</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
<td>0.174</td>
<td>2.133</td>
<td>81.704</td>
<td>-10.725</td>
</tr>
</tbody>
</table>

Note: $k_a$ and $k_d$ can be determined via matlab with three calculations for average.

5.3.3 Molecular interactions between fibroin and sericin

To further understand the interactions between fibroin and sericin, solution-state $^1$H NMR and FTIR were used to monitor the structural changes of fibroin protein and sericin protein in solution state.
Figure 5.6 $^1$H NMR spectrum of a 1 wt% silk fibroin and their hybrids solutions. (a) *A. pernyi*; (b) *B. mori*.

*A. pernyi* has significant amount of alanine (Ala) residues (50.5%) in its fibroin and serine (Ser) residues (23.49%) in its sericin, whilst *B. mori* has a high content of Ala (30.0%) and glycine (Gly) residues (42.9%) in its fibroin and a significant amount of ser residues (39.0%) in its sericin [36]. As shown in Figure 5.6a, *A. pernyi* fibroin has its characteristic peaks at around 3.9 and 3.6 ppm due to the $H_\alpha$ of Ala and $H_\alpha$ of Gly respectively. The $H_\beta$ of Ser as a shoulder peak appears at 3.53 ppm. After interacting with sericin, the $H_\alpha$ resonance of Gly moves up field to 3.53 ppm with more Ser involved. The enhanced adsorption for the methine proton at around 3.53 ppm in Ser indicates the formation of fibroin-sericin compound, resulted from the molecular interactions between Ser and Gly. In contrast to Figure 5.6a, a remarkable $H_\alpha$ resonance emerging at 3.4 – 3.7 ppm (Figure 5.6b) is mainly due to the $H_\alpha$ of Gly of *B. mori* silk fibroin, which overlaps the $H_\beta$ of the Ser residues. After sericin doping, the compound shows no obvious shift, indicating the chemical environment of the hydrogen atoms did not change.
Sericin of both *A. pernyi* and *B. mori* has high contents of Ser residues [232, 233]. Although *B. mori* fibroin has a high content of Gly (43 mol %), these residues are mainly in the repeated unit -(Gly-Ala-Gly-Ala-Gly-Ser)ₙ- which together with Ala form the crystalline region of fibroin [31]. This means the interactions between Ser residues on *B. mori* sericin and Gly residues on *B. mori* fibroin are mostly hindered (Figure 5.7). The primary structure of *A. pernyi* fibroin is quite different. It has hydrophobic poly -(Ala)₁₂- region that repeats alternatively with Gly to favor the formation of α-helix structure [84]. A fibroin molecule with α-helix conformation is more flexible which is conducive to the intermolecular hydrogen bonds formation between its Gly residues and Ser residues on sericin.

**Figure 5.7** Proposed reaction pathways for glycine and serine residues in fibroin and sericin from *A. pernyi.*
Interactions between molecules can be monitored by changes in their diffusion coefficient by \(^1\)H NMR. The self-diffusion coefficient of *A. pernyi* fibroin was \(0.52 \times 10^{-10} \text{ m}^2/\text{s}\) in D\(_2\)O solution, while that of sericin was \(0.97 \times 10^{-10} \text{ m}^2/\text{s}\). In their mixture, the observed coefficient \(D_{\text{obs}}\) (a combination of both the free and binding states) changed to \(0.49 \times 10^{-10} \text{ m}^2/\text{s}\) and \(0.56 \times 10^{-10} \text{ m}^2/\text{s}\) for fibroin and sericin, respectively. The self-diffusion coefficients of fibroin and sericin from *B. mori* were \(0.66 \times 10^{-10} \text{ m}^2/\text{s}\) and \(1.13 \times 10^{-10} \text{ m}^2/\text{s}\), respectively. In their mixture, the values \((D_{\text{obs}})\) changed to \(0.65 \times 10^{-10} \text{ m}^2/\text{s}\) and \(0.75 \times 10^{-10} \text{ m}^2/\text{s}\), respectively. The results demonstrated that the diffusion of sericin and fibroin from *A. pernyi* silk are hindered to a greater extent than those from *B. mori* silk, indicating a higher degree of complexation between sericin and fibroin from the wild silk. The diffusion coefficients of sericin-fibroin hybrids \(D_b\), *i.e.* in binding state only) are \(0.42 \times 10^{-10} \text{ m}^2/\text{s}\) and \(0.54 \times 10^{-10} \text{ m}^2/\text{s}\), for *A pernyi* and *B. mori* silk, respectively.

Since fibroin and sericin complex did not lead to extremely broadened NMR signals, the interactions between sericin and fibroin should be fast [234]. According to the model for first-order reversible fast exchange, the observed diffusion coefficient \(D_{\text{obs}}\) is the weighted average of the diffusion coefficients of molecules in binding state, \(D_b\) and in free state, \(D_f\) [234].

\[
D_{\text{obs}} = (1 - X_b)D_f + X_bD_b \quad \text{(Equation 5-7)}
\]

where \(X_b\) is the molar fraction of the molecules in binding sites; \(D_f\) and \(D_b\) are the diffusion coefficients in free state and in binding state, respectively.
Table 5.3 Diffusion coefficients \((D \times 10^{-10} \text{ m}^2/\text{s})\) obtained from PFG NMR experiments at room temperature \((20 \, ^\circ\text{C})\)

<table>
<thead>
<tr>
<th></th>
<th>(D_{\text{obs}})</th>
<th>(D_f)</th>
<th>(D_b) (^a))</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. pernyi</em></td>
<td>0.56</td>
<td>0.97</td>
<td>0.42</td>
</tr>
<tr>
<td><em>B. mori</em></td>
<td>0.75</td>
<td>1.13</td>
<td>0.54</td>
</tr>
</tbody>
</table>

\(^a)D_b\) is the diffusion coefficient of fibroin-sericin in binding state, which is assumed to be identical to the diffusion coefficient of the sericin molecules.

According to Equation (5-6) and the data shown in Table 5.3, \(X_b\) of sericin were calculated to be 0.75 and 0.64 for *A. pernyi* and *B. mori*, respectively. This suggested that 75 mol% of the *A. pernyi* sericin was associated with *A. pernyi* fibroin and 64 mol% of *B. mori* sericin with *B. mori* fibroin under the experimental conditions, further indicating a stronger affinity between sericin and fibroin from *A. pernyi* silk.

FTIR was also used to detect the molecular interactions between fibroin and sericin from both *A. pernyi* and *B. mori* silk. Figure 5.8 shows the FTIR spectra of fibroin and sericin proteins as well as their compound. *A. pernyi* fibroin has characteristic bands at about 1644, 1634, 1515, 1381 cm\(^{-1}\) and sericin at 1663, 1642, 1534, 1518 and 1398 cm\(^{-1}\) [36]. Fibroin and sericin of *B. mori* silk have peaks at around 1665 cm\(^{-1}\) and, 1535 cm\(^{-1}\), respectively [235]. These peaks were also evident on the *A. pernyi* spectra, showing similarity in structural conformation.
Figure 5.8 ATR infrared spectrum of the silk proteins in the region of 1750 cm\(^{-1}\)-
1350 cm\(^{-1}\). (a) \textit{A. pernyi}; (b) \textit{B. mori}.

A combination of the spectrum of the hybrid fibroin and sericin from \textit{B. mori} against
the spectra of pure fibroin and sericin indicates that fibroin and sericin of \textit{B. mori} silk
did not undergo observable structural changes when they were co-exist in solution. In
contrast, the characteristic peaks of \textit{A. pernyi} fibroin and sericin at 1644 cm\(^{-1}\) due to
the C=O stretching and NH deformation in amide I region and 1534 cm\(^{-1}\) in amide II region [236] shifted to 1627 cm\(^{-1}\) (parallel \(\beta\) strand) and 1524 cm\(^{-1}\), respectively, suggesting an increase in the intermolecular hydrogen bonding within the compound. The intensive hydrogen bonding between fibroin and sericin of \textit{A. pernyi} silk also explains on a molecular level the strong affinity of \textit{A. pernyi} sericin to fibroin.

5.3.4 Contact angle analysis

Contact angle can be used to characterize the affinity of a liquid to a substrate. The dynamic contact angles of the fibroin films against sericin solutions as a function of time is shown in Figure 5.9.

![Figure 5.9 Dynamic contact angles between sericin solution and fibroin films](image)

It can be seen from Figure 5.9 that the contact angle decreased as time increased. This could be due to substrate hydration, leading to decrease of contact angles [237]. Thus, the contact angles upon immediate contact (first frame of video) between the water
(with and without sericin) droplets and fibroin films are adopted here to illustrate the influence of sericin on the affinity of the droplets to the fibroin films.

**Figure 5.10** Snapshots of the side view of water/sericin solution droplet upon immediate adsorption with fibroin films

**Figure 5.10**a and b show that the contact angle between water and *B. mori* fibroin film is 99°, while that for *A. pernyi* film is 97°. This slight difference could be due to the small difference in the roughness of the two films. The roughness of *A. pernyi* fibroin film is 1.18 nm whilst that of *B. mori* fibroin film is 2.08 nm (Figure 5.11). The hydrophobicity of the films is also one factor that affect the contact angle. Nevertheless, the small difference in water contact angles indicates the overall surface properties of the two films are quite close. When sericin is present in the water droplet, the contact angles were changes to 95° and 75° for *B. mori* and *A. pernyi* fibroin films,
respectively. The drastic decrease in the contact angle between its sericin and fibroin film for the \textit{A. pernyi} indicates a greater affinity between its sericin and fibroin.

**Figure 5.11** Roughness maps of fibroin films based on a 5 um × 5 um region. (a) \textit{A. pernyi}; (b) \textit{B. mori}.

### 5.4 Conclusions

In summary, IGC studies demonstrate that the adhesion between sericin and fibroin in \textit{A. pernyi} silks is stronger and more heterogeneous than that in \textit{B. mori} silks. Compared to dispersive adhesion, specific adhesion plays a more remarkable role for total adhesion. QCM-D study enables a detailed investigation of the dynamic behaviors that occur during the interaction between fibroin and sericin. The highly negative $\Delta G$ value for \textit{A. pernyi} indicates the high adsorption affinity of its sericin to fibroin. The larger
Kₐ value and ΔD/Δf for *A. pernyi* silk indicate faster adsorption of its sericin on fibroin film. Studies based on NMR and FTIR show that, the adsorption of fibroin-sericin is a process involving interactions between glycine (on fibroin) and serine (on sericin), possible conformation changes between C=O stretching and NH deformation. These observations explain the difficulties in degumming *A. pernyi* fibres, which hinders the applications of them in the textile industry. However, the wild *A. pernyi* silk fibre exemplifies an excellent core-shell composite material found in nature. The rough interface between the two components, *i. e.* sericin and fibroin and their strong interactions facilitate strong adhesion between them, which may inspire the design of superior composites for various applications.
CHAPTER 6  Conclusion

6.1 Summary

This thesis examined the crystallite structure, mechanical properties, and thermal transport mechanism as well as adhesion property of *A. pernyi* silk fibres. The key findings and a recommendation for future study are provided in the following sections.

6.1.1 The mechanical properties of different silk fibres from *A. pernyi* cocoon

Silk fibres from three key parts of the *A. pernyi* silkworm, i.e. peduncle, outer floss and cocoon shell (both outermost and innermost parts) were studied in this work. Peduncle fibres showed well aligned arrangement, good plastic deformation and superior hysteresis effect to silks from other parts of the *A. pernyi* cocoon, which can effectively reduce the amplitude of cocoon swinging when it encounters physical attacks or strong wind. The high sericin content of outer floss contributes to some physiological properties such as anti-oxidant and UV protection of the cocoon. The outermost fibres have good hardness and elasticity, leading to their remarkable toughness to absorb the most breaking energy. Innermost fibres have superior elasticity therefore is shape-preservable and stable. The fibres from these parts contribute to the protective functions of the *A. pernyi* silkworm cocoon.

6.1.2 Crystallite structure versus mechanical properties

The relationships between nanocrystalline structure and mechanical properties were investigated. Heat treatment had an impact on the nanostructure formation of silk fibres. It can lower the tendency for peptide chains to self-assemble into β-crystallite
but promote the intramolecular β-sheet. The temperature-induced structural changes also affect the mechanical properties of fibres. Tensile measurements suggested that mechanical properties could be modulated by annealing the nanocrystalline structure. The stress and strain to failure decreased with increasing temperature and reached a minimum around 230 °C, whilst modulus and yield strength increased with temperature. The increasing intramolecular β-sheets can account for the superior modulus, while the average crystallite size has a positive effect on the yield strength. Compared to B. mori, A. pernyi silk fibres show more obvious temperature-dependent structural changes.

6.1.3. Crystallite structure versus thermal properties

The A. pernyi cocoon has an excellent thermal buffering function. The contributions of different cocoon components individually and in combination to thermal transfer were studied. The thermal conduction of raw cocoon and that with sericin and calcium crystals removed were investigated. Calcium crystals play a role in thermal transfer followed by sericin. Despite of the low thermal conductivity (0.05 and 0.03 mm²/s for inner and outer fibres respectively) in the transverse direction, the chain direction exhibited higher values of 0.35 and 0.2 mm²/s for inner fibre and outer fibre, respectively, from the A. pernyi cocoon. The low thermal expansion, high crystallinity and small pore size account for the relatively high thermal conduction of inner fibres. The 10-fold increase in the chain direction can be directly correlated to the intrinsic crystalline anisotropy, where weaker intermolecular interactions in the transverse directions can reduce phonon propagation.
6.1.4 The adhesion between fibroin and sericin

The differences of adhesion between fibroin and sericin from *A. pernyi* and *B. mori* were investigated to illustrate the reasons why *A. pernyi* needs more energy for silk degumming. The specific adhesion with a high degree of heterogeneity plays a leading role for the hard degumming of *A. pernyi* silk fibres. This specific adhesion arises from the intermolecular hydrogen bonding attributable to the glycine from fibroin and serine from sericin.

6.2 Recommendations for further work

6.2.1 The correlation of mechanical properties and crystalline structure at low temperatures down to subzero

The strength and toughness of *A. pernyi* silk fibres along with the increase of temperature have been revealed in terms of the nanocrystalline structure modification. As the *A. pernyi* silk cocoon is typically found in the northeast part of China, where temperature is very low, especially down to -30 °C in winter, further studies need to be done to understand the mechanical properties of *A. pernyi* silk fibres and hence the mechanical properties and protective function of the cocoon in extremely cold weather.

6.2.2 The mechanical properties of fibroin and sericin

The raw silk fibres and degummed silk fibres have been tested for their mechanical properties. Sericin layer, due to its low content, is mostly neglected for its effect on the mechanical property of the fibre. Fibroin fibre is composed of nanofibrils organized into a bundled structure to form fibroin filaments. The fibroin filaments are glued by sericin protein containing high levels of serine and tyrosine. An understanding of the difference of mechanical properties between fibroin and sericin
will shed light on the role of various structural and chemical components of a silk fibre in providing mechanical strength of the fibre. This is important for biomimicking biological structures to design protective materials.

6.2.3 The role of calcium ions in the interactions between fibroin and sericin

The calcium crystals, mainly composed of calcium oxalate monohydrate, are deposited on *A. pernyi* silk fibres, particularly on the outer layers of the cocoon. This kind of crystal is originated from the excrement of *A. pernyi* silkworm. It is formed in the process of silk spinning. The main element of this crystal is calcium ion. From many literatures, the salt ions like calcium ion can affect the conformational structure and ionic charge of proteins, which certainly will affect the interactions between fibroin and sericin. Studying the role of calcium ion in the interaction between fibroin and sericin is essential to understand the formation of calcium crystals and the adhesion process of fibroin and sericin.

6.2.4 Thermal conduction of silk fibres with low temperatures down to -30 °C

Thermal conduction above room temperature has been studied in this project. As *A. pernyi* silkworm can survive in a harsh outdoor environment with temperatures as low as -30 °C, it is essential to understand the thermal conduction of silk fibres at the low temperatures in order to understand the excellent thermal regulation properties of the cocoon.

6.2.5 The effect of moisture/humidity on the crystalline structure and corresponding thermal property

The effect of temperature on the crystallite and thermal transfer has been investigated in this work. As an important environment factor, moisture/humidity shall have effects
on the thermal properties of silk fibres. A good understanding of moisture/humidity effect on thermal property of silk fibres is needed to get further insight into the role of the cocoon as a natural defence for the pupae. As studied in chapter 4, cotton and ramie have relatively high thermal conduction, which may be partially due to their superior moisture absorption. Hence moisture/humidity may play a role in tuning the thermal property of *A. pernyi* silk fibres to affect the thermal regulation function of the wild cocoon.

6.2.6 Modelling the thermal transfer of crystallites, intra-molecular \( \beta \)-sheets and the amorphous phase

Thermal property of silk fibres has been studied in chapter 4. As illustrated in chapter 3, *A. pernyi* silk fibres are composites with heterogeneous structures consisting of crystallites, intra-molecular \( \beta \)-sheets, amorphous region and pores. These multiple components affect the thermal conduction of a whole fibre. To isolate the contribution of each part, modelling the thermal conductivity/diffusivity of each part, *i.e.* crystallites, intra-molecular \( \beta \)-sheets and amorphous structure, is essential to tailor the thermal property of this semi-crystalline fibres by the proper choice of constituents and configuration.
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