Hagfish Slime:
Fine-Tuning the Mechanical Properties of a New High Performance Fiber

By

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B.Sc. Integrated Sciences, University of British Columbia, 2001

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE STUDIES

(Zoology)

THE UNIVERSITY OF BRITISH COLUMBIA

July 2005

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ABSTRACT

The race to find new high performance materials is at an exciting stage. Science is in the midst of attempting to investigate any and all materials that are present in the world with the hope of finding superior, cheaper, environmentally friendly materials. Nature, it seems has been unknowingly at the race for quite some time, and is leading it in some areas.

Intermediate filament-based materials promise good mechanical characteristics with the added benefit of self-assembly. Although much is known about the mechanical properties of other intermediate filament-rich materials such as wool, those materials are not purely composed of intermediate filaments and usually have added complexities in terms of synthetic manufacturing. This thesis focuses on manipulating and understanding the relationship between structure and function of essentially pure intermediate filament-based hagfish slime fibers.

Previously described α-helix ↔ β-sheet transition in the coiled-coil domains of hagfish fibers' intermediate filaments subunits was quantified using a novel in vitro light microscopy technique. This allowed for optimization of draw processing techniques that lead to improved tensile mechanical properties. Improvement was achieved via formation of a β-sheet crystal network in the draw processed fibers.

Dimensional stability was achieved via physical and chemical processing and resulted in a new candidate environmentally friendly, high-performance fiber.
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1 INTRODUCTION

1.1 The Search for New Materials

Commonly used high performance materials such as Nylon, Kevlar®, and various rubbers are so widely used that their commercial production is sometimes in excess of 1 km of material in 1 minute, at a single factory. Although these materials possess amazing and adjustable properties and are cheap to produce, they are relatively harmful to the environment: Their production usually involves toxic stages; they are not biodegradable and are not made of easily renewable resources.

The search for less environmentally invasive materials has been an ongoing one. The challenge faced is finding more natural materials that could rival the properties of the synthetic ones. Figure 1 shows typical tensile test mechanical data for Kevlar® and rubber fibers. The data are presented as a stress-strain curve, where stress (σ) is the normalized force (F), defined as $\sigma = \frac{F}{A}$, where A is the initial cross-sectional area of the fiber. The strain (ε) is the normalized deformation, defined as $\varepsilon = \frac{\Delta L}{L_0}$, where $L_0$ is the fiber's initial length, and $\Delta L$ is the change in fiber length. The slope of the initial part of the stress-strain curve gives the stiffness ($E_{\text{int}}$) of the material, and the maximum values of stress and strain at the point where the fiber fails give the strength ($\sigma_{\text{max}}$) and extensibility ($\varepsilon_{\text{max}}$) respectively. The area under the curve gives the energy required to break the material and for fibers provides an indication of toughness. Note that Kevlar® has a very high tensile strength, and as a consequence it is commonly used to make very high-end fibers that are woven into bullet proof vests. On the other hand, rubber is not as strong, but it is an extremely extensible material that can be deformed time and again without losing this property. Kevlar® and rubber represent two extremes of a wide continuum of mechanical properties. A hypothetical material (Figure 1) that could exhibit high stress tolerance and could be stretched to high stains would therefore be very tough, and would be much
sought after; even more so if it could selectively exhibit a whole range of such properties. Far more desirable would be such a material that has a low impact on the environment.

![Graph of mechanical properties of Kevlar®, a typical rubber, and a hypothetical high-performance material.](image)

**Figure 1.** A representation of the mechanical properties of Kevlar®, a typical rubber, and a hypothetical high-performance material. $\sigma_{\text{max}}$ and $\varepsilon_{\text{max}}$ represent the breaking strength and extensibility of the fiber respectively. Stiffness ($E_{\text{int}}$) is the slope of the graph – showing Kevlar® to be very stiff. Toughness (shaded area) is represented by the area under the stress-strain curve. Note that both Kevlar® and rubber are not very tough (the area under their curve is not very large). Y axis represents stress, which is the normalized force per unit initial area ($\sigma = F/A_0$). X axis represents the strain, which is the change in length over initial length ($\varepsilon = \Delta L/L_0$) (Gosline et al. 1999).

### 1.2 Biological Materials

Nature, it seems, has been in the race to produce such hybrid high-performance fibers far longer than humans, and in many ways is winning. Structural proteins such as silks and intermediate filament-based materials exhibit a wide range of
properties and are truly environmentally friendly. Gosline et al. (1999) demonstrate the potential in spider dragline silk (Figure 2). From their research it is possible to imply that spider silk is essentially modified by the spiders to produce materials that are superior in some aspects to any man-made high performance materials. This silk can be as extensible as most rubbers and is stronger, whilst essentially the same silk can be modified by the spiders to produce a material that almost rivals Kevlar® in tensile strength, but makes up for the difference in toughness. Figure 2 and Table 1 demonstrate this potential. Although spider dragline silk has a lower breaking stress than Kevlar®, its main advantage is its ability to strain – this advantage leads to much greater toughness than either rubber or Kevlar®.

![Graph](image)

**Figure 2.** Spider silk – a protein-based biological material – is a worthy rival to some of the world’s best man-made fibers. This representation is adopted from Gosline et al. (1999) in attempt to display the wide range of properties spider silk displays. Note the much larger area under the curve of dragline silk than that of Kevlar®, or rubber.
Table 1 compares the mechanical properties of spider silk to those of Kevlar® and rubber. The enormous toughness that spider silk possess leads to the continual attempts to mimic this material synthetically (Winkler et al. 1999).

Table 1. Tensile mechanical properties of spider silk and other materials. Note the much higher toughness (ability to absorb energy) of spider dragline silk (Gosline et al. 1999).

<table>
<thead>
<tr>
<th>Material</th>
<th>Stiffness $E_{\text{int}}$ (GPa)</th>
<th>Strength at Break $\sigma_{\text{max}}$ (GPa)</th>
<th>Extensibility $E_{\text{max}}$ ($\Delta L/L$)</th>
<th>Toughness (MJ/m$^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kevlar®</td>
<td>130</td>
<td>3.6</td>
<td>0.03</td>
<td>50</td>
</tr>
<tr>
<td>Rubber</td>
<td>0.001</td>
<td>0.05</td>
<td>3.5-10</td>
<td>70</td>
</tr>
<tr>
<td><em>Spider Dragline Silk</em></td>
<td>10</td>
<td><strong>1.2</strong></td>
<td><strong>0.3</strong></td>
<td><strong>160</strong></td>
</tr>
</tbody>
</table>

This amazing toughness is thought to stem, in part, from the specific and highly repetitive structure of the spider dragline silk (Gosline et al. 1999). The silk is comprised of multiple repeats of identical blocks of polypeptide sequences that are thought to interact with each other, forming $\beta$-sheet networks as a consequence. These $\beta$-sheets are rich in hydrogen bonds and thought to lead to the silk’s great strength and toughness. One of the inherent problems with the structure of spider silk, however, is the liquid crystal phase it must go through to be properly spun (Vollrath and Knight, 2001). This and other limitations create major hurdles in trying to artificially mimic this material, and progress in this area seems to have halted.
1.3 Intermediate Filament Based Materials

Hard keratins, such as horse hooves and wool, have been shown to be very durable and to possess very high toughness (Kasapi and Gosline, 1997; Hearle 2000; Fraser, 1972). The entire process seen in Figure 3 is spontaneous. These materials are comprised of α-helical subunits (Figure 3 (A)) that make up the coiled-coil domains of protein dimers (Figure 3 (B)). These pair up and self-assemble serially into proto-filaments (Figure 3 (C)) that in turn form groups of four and self-assemble into proto-fibrils (Figure 3 (C)). Four of these proto-fibrils interact to form an intermediate filament (Figure 3 (D)) that is then imbedded in a cross-linked protein matrix (Figure 3 (E)). The structure of hard keratin is thought to convey in part its remarkable properties. This intermediate filament based material is very attractive in terms of its mechanical properties, and even more attractive in its amazing ability to self-assemble within living cells (Perry and Steinert, 1999).
Figure 3. The structure of wool. A-Helical proteins (A) twist together to form dimers that have coiled-coil domains (B). These form individual proto-filaments (C) that assemble into proto-fibrils (C), that form the intermediate filaments (IF) (D), which are then embedded in a cross-linked matrix to form the mature hard keratin (E) (figure adopted from Parry and Steinert, 1999; Hearle, 2003; and Fudge and Gosline, 2004).
Figure 4 shows data from Hearle (2000) for wet wool fibers that are plotted along side spider dragline silk data from Gosline et al. (1999). At first glance the wool mechanics seem quite unimpressive when compared to spider silk. However, the hidden possibilities their structural design and self-assembly ability hold are intriguing. Table 2 shows wool fibers to also be worthy of examination when compared to Kevlar®, rubber and spider dragline silk.

![Graph showing stress vs. relative strain for Spider Dragline Silk, Dry Wool, and Wet Wool](image)

**Figure 4.** At first glance, wet or dry wool fibers seem unimpressive when compared to spider silk. Yet, they hold a structural and morphological commonality to many other materials that may possess hidden mechanical secrets. Y axis represents stress in MPa, X axis lower strains than previous figures (Fudge et al. 2003; Gosline et al. 1999; Wainwright et al., 1976).
Table 2. Tensile Mechanical Properties of wool compared to other materials. Wool is not as impressive as spider silk, but is as tough as Kevlar® (Gosline et al. 1999; Wainwright et al., 1976).

<table>
<thead>
<tr>
<th>Material</th>
<th>Stiffness $E_{\text{int}}$ (GPa)</th>
<th>Strength at Break $\sigma_{\text{max}}$ (GPa)</th>
<th>Extensibility $E_{\text{max}}$ (ΔL/L)</th>
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<tr>
<td>Spider Dragline Silk</td>
<td>10</td>
<td>1.2</td>
<td>0.3</td>
<td>160</td>
</tr>
<tr>
<td>Wet Wool</td>
<td>0.5</td>
<td>0.2</td>
<td>0.5</td>
<td>60</td>
</tr>
<tr>
<td>Dry Wool</td>
<td>4</td>
<td>0.25</td>
<td>0.3</td>
<td>60</td>
</tr>
</tbody>
</table>

Successfully harnessing materials such as spider silk for commercial use, or improving the properties of wool would truly be a major accomplishment. Although much research is done in the area of these materials, there also seems to be little progress to successfully mimic, produce and manipulate such materials. Many other materials are comprised of the same intermediate filament building blocks as wool, so it may be necessary to investigate those materials as they may open new venues for possibilities of high-performance fiber advancement.

In spite of the wide range of literature on the properties of keratin-based materials like wool, until recently virtually nothing was known about the mechanical properties of intermediate filaments themselves. Recently, however, it has been discovered that hagfish produce fiber reinforced slime, in which the slime threads are made of essentially pure intermediate filaments. This opened up new opportunities for the study of these materials.
1.4 Hagfish Slime Fibers: A Model for IF-Based Materials

Fudge et al. (2003, 2004) present compelling data on hagfish fibers. These fibers are composed of IF proteins, which are essentially identical to those found in wool, and they exhibit remarkable mechanical properties. Figure 5 and Table 3 show the tensile mechanical properties of a wet hagfish slime fiber. Note the very low initial stiffness (initial modulus), the impressive increase in the slope of the curve due to strain hardening, and the amazing extensibility of this fiber – giving the fiber toughness easily comparable to that of spider dragline silk ~130 MJ/m³. Yield will be discussed below.

![Figure 5](image)

**Figure 5.** This typical hagfish fiber stress-strain curve shows that the fiber possesses impressive extensibility and toughness (Fudge and Gosline, 2004). Note the ca. 40x increase in slope due to strain hardening. Insert focuses on the initial stiffness of the fiber and on the yield zone (change in slope from I to II) – this will be discussed below.
Table 3. Tensile mechanical properties of wet hagfish fiber compared to mechanical data of spider dragline silk and wool (Fudge et al., 2003; Gosline et al., 1999; Wainwright et al., 1976). Hagfish fibers display much lower (100x) initial stiffness than its keratin relative – wool, but much higher extensibility and toughness.

<table>
<thead>
<tr>
<th>Material</th>
<th>Stiffness $E_{int}$ (GPa)</th>
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<td>Wet Wool</td>
<td>0.5</td>
<td>0.2</td>
<td>0.5</td>
<td>60</td>
</tr>
<tr>
<td>Dry Wool</td>
<td>4</td>
<td>0.25</td>
<td>0.3</td>
<td>60</td>
</tr>
<tr>
<td>Wet Hagfish Fiber</td>
<td>0.005</td>
<td>0.2</td>
<td>2.5</td>
<td>130</td>
</tr>
</tbody>
</table>
1.4.1 About Hagfish and their Slime

Hagfish are bottom dwelling marine chordates. Their main specialty and the origin of interest of this thesis is their ability to produce copious amounts of slime when startled, probably as a defense mechanism. They have numerous specialized glands located on either side of their abdomen (Figure 6 (A)) (Ferry, 1941; Jensen, 1966). These glands contract to expel their crude slime content, which rapidly hydrates to a final polymer concentration of about 0.004% (Fudge and Gosline, 2000), thus enabling the hagfish to leave a large sphere of slime behind it, potentially to distract its attacker. The protective sphere is reinforced with fibers. These fibers start off as miniature bundles (Figure 6 (B, C)) containing one continuous fiber (ca. 15 centimeter long), and upon expulsion these fibers unravel.

![Image of hagfish with slime](image1.jpg)

**Figure 6.** An anesthetized hagfish with crude slime that had been expressed from two of its slime glands (A). Thousands of the tiny GTCs (B, C) are present in a drop of this crude slime (Fudge et al., 2003; Koch et al. 1994).

Looking further into the structure of one of these tiny fiber bundles reveals something amazing. The bundle is one continuous fiber made up entirely of intermediate filaments (Fernholm, 1981). This provides an excellent system for
studying intermediate filaments independently of other structural components (Downing, 1984). Wool, as stated above, is made up of intermediate filaments embedded in a matrix; hagfish fibers allow us to study the mechanical properties of intermediate filaments that are not embedded in anything.

The formation of these fiber bundles is a marvel on its own. Figure 7 summarizes the important steps. Individual α-Helices, similar to those described by Hearle (2000), form coiled-coil dimers (A) that self-assemble into sub-filaments, that in turn form complete (10 nm in diameter) intermediate filaments (B) that align to form one continuous macroscopic fiber (Koch et al., 1994). This process occurs entirely within a single Gland Thread Cells (GTC) and does not stop until the entire cell is completely filled with this newly formed fiber (C) (Downing, 1981b). Upon ejection out of the glands, the individual GTC lose the thin cell membrane that coated them (D, E).

**Figure 7.** The structural hierarchy of hagfish slime threads. From an individual coiled-coil subunit (A), to a self-assembled intermediate filament (B), to a maturing Gland Thread Cell (GTC) (C), to a fully mature, ejected GTC (D, E) (Downing et al. 1981; Koch et al. 1994; Fudge et al. 2003).
1.5 Fiber Structure

These fibers provide us with a system for studying Intermediate Filaments on the macromolecular level (Terakado et al. 1975). However, this transition to the macromolecular level may carry with it unwanted artifacts. Single intermediate filaments may behave very differently than intermediate filaments that are interacting in a group – there may be some synergetic effects, or some hindering effects.

Wet wool fibers exhibit complete elastic recovery at all degrees of deformation (Gupta and Rao, 2003). Hagfish fibers behave very similarly at low strains (Figure 8(A)), but begin to display odd behaviors at strains greater than 0.35. Figure 8 (B), shows the presence of a yield zone ($\sigma_{yield}$) (a change of slope in the stress-stain curve) in wet hagfish fibers at a strain of 0.35. It became evident that the specific structure of the fibers is responsible not only for the impressive toughness of the fibers, but also for this interesting yield phenomenon (Fudge et al. 2003). The key feature of post yield deformation is that the fibers do not recover more than 35% of the strain they experience. This is unlike wool fibers that recover to their initial strain ($L_0$) completely, regardless of the extension they are deformed to. Figure 8 (C) shows the final strain hagfish fibers recover to after some initial maximum strain. The only exception is at strains below 0.35 (~35%) where complete recovery is seen (as in Figure 8 (A)). This novel characteristic of irreversibility – was measured for the 10 minutes following deformation of the fibers (Figure 8 (C)). Although hagfish fibers are composed of almost entirely intermediate filaments and are therefore made from similar subunits to those that make up wool (Koch et al. 1991; Fudge and Gosline, 2004), they do not seem to recover – their deformation appeared to be a permanent one.
Figure 8. Fudge et al. (2003) demonstrate that below a strain of 30% - the fiber behaves in a elastic manner – recovering to it initial length immediately (A). When taken above the 30% extension range, the fiber is only able to recover 30% of the deformation they receive (B). This appears to be consistent regardless of what maximal deformation (strain) the fiber undergoes (C). Note the yield point (B) – some structural change must be occurring in the fiber at that point.
These results led to the development of a model for the coiled-coil dimers that make up the intermediate filaments to try and explain some of the results observed. Figure 9 shows Fudge et al.'s model of the dimers. Coiled-coil domains (region 1) are flanked by amorphous terminal domains (region 2). These amorphous domains can be easily extended up to strains of 0.35 (35% extension) (Figure 10) and upon release recoil back to their original dimensions (Figure 9). These amorphous domains were integrated into the model in attempt to explain the low initial modulus observations seen above (Table 3). The terminal domains act via a series force transfer, resulting in sliding of the coiled-coil domains relative to adjacent ones (Figure 10) – inhibiting hydrogen bond formation between adjacent coiled-coils.

**Figure 9.** Fudge et al.'s Model of the dimers of coiled-coils (region 1) with amorphous terminal domains (region 2) that make up the hagfish intermediate filaments (2003). Sliding of the coiled-coil regions (Region of Sliding) relative to other coiled-coils in response to small deformations (<35% extension) as modeled by Fudge et al. Because the terminal domains (region 2) are elongated, the coiled-coils are less overlapping, inhibiting hydrogen bond formation between adjacent coiled-coils (Fudge et al. 2003). This explains the low initial modulus data seen above (Table 3).
1.5.1 The $\alpha \rightarrow \beta$ transition

If deformation by extension continues past the initial 35%, the coiled-coil domains start to undergo a conformational change and it is this conformational change that creates the yield behaviour of the fiber (Figure 8 (B)). This opens possibilities for physical stabilization of the mechanical properties. As the fibers stretch the $\alpha$-helices that form that rigid domain, start to uncoil, and with continued extension the proteins begin to form $\beta$-pleated sheets (Fudge et al., 2003). These $\beta$-sheets may then be able to interact and lead to new fiber characteristics. Figure 10 illustrates the possibilities for increased inter-protein hydrogen bond formation upon deformation of $\alpha$-helices into $\beta$-pleated sheets (Figure 11 also illustrates this ability). The $\alpha$-helix in Figure 10 is only able to form hydrogen bonds within the helix, not with neighboring helices. Conversely, the $\beta$-sheets in Figure 10 are able to form hydrogen bonds with adjacent $\beta$-sheets, providing opportunities for extensive 3D $\beta$-sheet crystal formations. Figure 11 illustrates the transition within the coiled-coil domain of a single intermediate filament dimer. This $\alpha$–helix $\rightarrow$ $\beta$-sheet transition was supported with x ray diffraction patterns as well as Congo Red staining procedures (Figure 12) (Fudge et al., 2003).
**Figure 10.** $\alpha$-helices (Left) can deform into $\beta$-sheets (right). When this happens the $\beta$-sheets can form more hydrogen bonds with adjacent $\beta$-sheets (Picture from Zubay, 1998).

**Figure 11.** Representation of the uncoiling of the $\alpha$-helical coiled-coil regions of a single hagfish fiber intermediate filament subunit (dimer) into $\beta$-pleated sheets (Fudge et al. 2003).
Figure 12. X ray diffraction (I) and Congo Red Staining (II) evidence in support of the $\alpha \rightarrow \beta$ transition that occurs as the hagfish fibers are deformed. Note the increasing presence of $\beta$-sheets as the fibers are deformed to greater extensions (from $\varepsilon = 0.0$ to $\varepsilon = 1.0$). The transition in fibers strained to 100% of their original length (image I-C) is matched by the results of the Congo Red stain (II-E) (Fudge et al., 2003).
1.5.2 Mechanical Consequences of Structural Changes

Fudge et al.'s model also serves to explain the much lower 5 MPa initial modulus of hagfish fibers (Figure 5 insert) than the 500 MPa of wet wool (Table 2). Fudge et al.'s modeled terminal domains that can be easily deformed and recover elastically (Figure 9) and therefore result in a low initial modulus. However, once past that initial 35% extension the coiled-coil dimer domains of the model begin opening – forming β-sheets (Figure 11) and the final yield stress is equivalent to that of Hearle’s wet wool fibers (~200 MPa).

This α → β transformation opens possibilities to creating a biological based material that could potentially rival synthetic high performance fibers, while circumventing the problem of a transient, liquid-crystalline phase faced by the spider silk researchers. It is possible that once the coiled-coils pop open they can, once again, slide relative to adjacent ones – but this time the sliding is in the reverse direction, allowing increased overlap of the coiled-coil domains – increasing the likelihood of hydrogen bond formation – thus leading to stabilization of the new β-sheet crystals. It is this process that will be the focus of this thesis. In order to create a new tunable, biologically-based material, this thesis investigates the relationship between structure and function of hagfish fibers, and a method to quantify these changes is implemented. Because superior tensile properties are seen in Fudge et al.'s (2003) data at strains around 1.5, where strain hardening seems to occur, this investigation will focus on that extension as a target for stabilization and optimization of formed β-sheet crystals.

1.6 Create a New Material

Although Fudge et al. (2003) did show that the fiber becomes somewhat stable post deformation, the inconsistency with Hearle’s wool fibers (2003) is striking. It is quite possible that the recovery time scale is greatly changed due to structural differences in the two fibers. Hagfish fibers lack the reinforcing protein matrix that surrounds the intermediate filaments in wool. It is possible that this
matrix stabilizes the wool fibers, enabling them to recover following a deformation.

Since a new high performance material is sought, the investigation will lead into methods to stabilize newly acquired properties that are created when hagfish fibers are draw-processed into β-sheet stabilized materials. It is desired to create a material that has adjustable characteristics, but it is equally important to fix these new characteristics temporally as well as spatially.

It will be also important to attempt to make the fiber stable regardless of the environment it will encounter. The first step will be to isolate the fibers and remove them from their natural habitat – dehydrate them – and measure the change in their properties. It is a necessity to work through a wet phase since that is the form the fibers are collected while in their packaged state.

### 1.7 Structure and Function – Function and Structure

Before testing novel mechanical properties, the potentially tight relationship between structure and function must be investigated. Gosline et al. (1999) discuss the importance of β-sheet crystal formation as the result of the processing of silks. They demonstrate that the specific polypeptide sequence of a given silk fiber is the means in determining how that fiber will behave mechanically. Specific sequences allow for the formation of these β-sheets in one fiber, but small differences in the sequence lead to a very different formation in another. This could very well be true in the case of hagfish fibers.

It is assumed that all hagfish fibers are created equal and that all are uniform in all aspects. This allows for investigation into the nature of this structure versus function relationship. One of the goals here will be to understand that link, and attempt to quantify it.
1.7.1 Hypotheses

Modification of properties by deformation and by physical and chemical stabilization is measured in tensile strength and deformed strain of the processed fibers. These data are later contrasted to previous research in order to demonstrate a significant change in mechanics and potential versatility.

This thesis will test the following hypotheses:

1. **Assuming the \( \alpha \)-helix \( \rightarrow \beta \)-sheet transition in deformed hagfish fibers does occur, hagfish intermediate filament coiled-coil domains exhibit a reversible \( \alpha \)-helix \( \leftrightarrow \) to \( \beta \)-sheet transition that could be made permanent via physical or chemical manipulation.**

2. **Assuming the \( \alpha \)-helix \( \rightarrow \beta \)-sheet transition is a real one, structurally changing hagfish fibers will have a direct influence on changing their material properties.**

In order to test **hypothesis 1**, a series of experiments will be presented to demonstrate the reversibility of the \( \alpha \rightarrow \beta \) transition and the subsequent physical and/or chemical manipulations that must take place to make this transition a permanent and stable one. Also a new method for quantifying the degree of deformation and stability of the processed fibers will be presented.

In order to test **hypothesis 2**, a series of tensile mechanical tests, similar to those carried by Fudge et al., will be presented in support of the newly acquired improved mechanical properties that result from the various physical and chemical manipulations used to test hypothesis 1.

1.7.2 Measuring Structural Change (in vitro)

Fudge et al. (2003) describes structural changes in hagfish fibers, resulting from deformation due to processing, by using established methods such as X-ray diffraction and Congo Red staining. These are relatively reliable and accurate methods, but they carry a very large cost to them – the fiber is destroyed during
the process of investigation (Puchtler at al. 1985), and the isolation procedures required probably create artifacts in the data. This thesis will attempt to modify existing real-time methods of quantifying structural change.

Birefringence is the double refraction of light, or the division of light into two rays (the ordinary and the extraordinary) when it passes through an anisotropic material and is dependent on the polarization of light (Wood, 1964). It is therefore proportional to the degree of crystalline organization of a given material – the more crystalline it is, the greater the refraction, and therefore, the greater the birefringence. Birefringence has been used in the past to measure degree of β-sheet crystal formation (Puchtler et al. 1985) and appears to be a relatively simple, quick way that allows fairly accurate determination of increase in crystal structure, and therefore β-sheet formation. It’s major advantage being its ability to investigate hydrated samples and its ability to track changes with strain and time in real-time. Therefore a single fiber can be tracked through many different conformational changes in response to deformation without the need to fix the fiber and dehydrate it. By focusing on a single fiber, the error due to variation among fibers is significantly reduced, as well as no artifacts are formed in the process by circumventing the need to dehydrate and fix the fiber.

1.7.3 Stabilization of Properties

The attempt to create a new stable, tunable material requires physical manipulation of the fiber. The primary method for physical stabilization of the processed fibers will be draw-processing (i.e. extension beyond the yield point) followed by annealing. This method is simply temporally varying the extent of potential hydrogen bonds among the deformed coiled-coil domains and among the terminal ends of the dimers. Simply allowing newly released (due to deformation) α-helices the time to interact with each other to such an extent that the fiber will not recover and will maintain its new characteristics because of the formation of more extensive β-sheet structure.
It is possible that physical manipulation alone is insufficient for a high degree of stabilization. To investigate this, chemical manipulation is carried out. This involves covalently cross-linking the plentiful lysine amino-groups that make up a typical hagfish fiber (Fudge et al., 2002) using glutaraldehyde – a common protein cross-linker (Zhang et al., 2003).

1.7.4 Mechanical Consequences of Processing

The overall purpose of this investigation is to create a new, environmentally friendly, high-performance fiber. Several different methods and techniques are used to modify and stabilize the hagfish fiber’s existing properties. Extra care is taken to ensure these new properties are stable - will not degrade over time. What remains is to demonstrate that all these improved manipulations and techniques have actually created a fiber that could rival some of the advanced synthetic materials that are commercially available. A good measure of success is the comparison of the new properties to those of spider dragline silk – the much sought after “holy grail” of biomaterial engineering. To demonstrate the new tensile mechanical characteristics of the processed hagfish fibers, a series of micro-mechanical tests is presented.
2 MATERIALS AND METHODS

2.1 Experimental Animals

Pacific Hagfish (*Epratretus Stoutii*) were captured at approximately 200 m off Wizard Island, Barkley Sound, British Columbia at a depth of approximately 100 m. Traps were baited with putrid pork chops and left for several hours, or over night. Hagfish were transported, chilled and partly sedated (400 mg/l tricaine methanesulfonate until somewhat unresponsive to the touch, then rinsed several times with clean seawater), back to the University of British Columbia where they were held in a 400 l aquarium of chilled artificial seawater (Instant Ocean at ~35%, 9°C) according to guidelines and regulations set by the UBC committee for Animal Care (Protocol #A02-0003).

2.2 Slime Collection

Crude slime was collected similarly to methods previously described by Downing et al. (1984) and Fudge et al. (2000). Hagfish were anaesthetized in a 4L anesthetic solution (375 mg/l tricaine methanesulfonate, 625 mg/l sodium bicarbonate, Instant Ocean at ~35%, 9°C). Once unresponsive to touch, they were placed atop a wet disposable cloth in a dissection tray. They were then rinsed several times with dH$_2$O and blotted dry using Kimwipes®. Crude slime was extruded out of slime glands in the rinsed area using a short, local, mild electrical stimulation of the surrounding muscles (8 V, 14 milliseconds, 80 Hz using a Grass Medical Instruments model S6 stimulator). The extruded crude slime was immediately placed in a high osmolarity citrate buffer solution (0.9 M sodium citrate, 0.1 M PIPES, pH 6.7) in accordance with Fudge et al. (2000).
2.3 Micromechanical Testing

Stress – Strain measurements of hagfish slime fibers were taken using a modification of a glass micro-beam force transducer described by Gosline et al. (1994) and Fudge et al. (2003). Measurements of the glass beam’s deflection under a microscope are translated into force values using the beam theory equation as described in Fudge et al. (2000).

Individual fibers were transferred in their native packed form into a dH$_2$O filled testing chamber (Figure 13 (B)) using a sharpened toothpick. The fibers were allowed sometime to unravel (ca. 10 minutes), then they were tied at both ends to vertical stainless steel rods (ca. 0.3 mm) mounted stationary at one end and on a movable platform controlled by a micrometer (movement restricted to one dimension). To secure the fibers they were wrapped around their glass or steel target several times, then carefully tied using square knots. Fibers then underwent their specific treatment followed by a dehydration process. This dehydration involved serially replacing the solution in the chamber with 100% ethanol. Ethanol stiffens the thread (Fudge et al. 2003) and provided a lower surface tension, thus minimizing the risk of further processing upon removal into air. The level of ethanol was lowered until it was possible to pop the fibers through the surface using a single human eyelash attached to a long needle. This removal process allowed for absolute fixation to the stainless steel post on the movable platform and to the glass rod using cyanoacrylate surface-insensitive gel (Loctite® #454).
During the measurement cycle of the experiment fibers were strained at a rate of 0.096 mm/s ± 0.002 by connecting the movable platform’s micrometer to a 72 rpm motor (Superior Electronic type SS25) via a flexible rubber belt and a manufactured plastic pulley. Strain refers to the change in fiber length divided by the initial fiber length ($\varepsilon = \frac{\Delta L}{L_0}$).

A video camera mounted on a Wild microscope with a low power objective (4x) allowed a video dimension analyzer (VDA model 303, Instrumentation for Physiology and Medicine, San Diego) to track the glass micro-beam deflection, and the resulting voltage output from the analyzer was collected at 20 Hz using a National Instruments DaqPad 4060E input/output board and Labview v. 6 data collection software (National Instruments, USA). The VDA/DaqPad apparatus was calibrated by following the voltage change versus distance moved using a B&L Calibration Micrometer slide.
Force was calculated based on the idea that very small forces can be measured by tracking the bending of a thin glass beam as the fiber is being stretched. These beam deflections can be converted into force values by using the equation derived from beam theory:

\[ F = \frac{3dEI}{l^3} \]

Where \( F \) is the force, \( d \) is the beam deflection, \( E \) is the young's module of glass \((5.72 \pm 0.06 \times 10^{10} \text{N/m}^2)\), \( I \) is the second moment of the area of the beam and \( l \) is the length of the beam from the anchoring point to the point of fiber attachment. This relationship holds for small deflections of about 10% of the beam length. Most of the deflections recorded were of the order of 1%.

*These glass beams were not always perfectly constant along their length. The second moment of area for a uniform cylinder with radii \( r_1 \) and \( r_2 \), where \( r_1 \) is the radius at the point where the beam is fixed and \( r_2 \) is the radius at the point of fiber attachment is:

\[ I = \frac{\pi}{4r_1^2r_2} \]

Stress was calculated as \( \sigma = \frac{F}{A_0} \), where \( A_0 \) is the initial cross sectional area of the fiber – measured using scanning electron microscopy (SEM) as described below.

Strain was calculated from the time that samples were collected and related back to the pre-measured micrometer/motor speed and the initial resting length of the mounted thread. Initial Length was measured using modified calipers with fine needles tips, allowing more precise measurements. These strain data were also corrected for the deflection of the glass micro-beam.
2.3.1 Determination of Thread Diameters for Micromechanical Testing:

Thread diameter was obtained by keeping a small snippet of the fiber, mounting it onto a SEM stub. The sample was then measured under a Hitachi S4700 scanning electron microscope (ca. x15000 magnification) at both ends and at the centre of the snippet. 5 measurements were taken at each location and the overall average of the 15 measurements was used. Fibers were assumed to perfectly cylindrical with a cross-sectional area of $A = \pi r^2$.

2.4 Uncertainty of Micromechanical Testing

As described in Fudge et al. (2003), the uncertainty of the four variables used is combined in the following equation:

$$\frac{u_F}{F} = \sqrt{\left(\frac{u_l}{l}\right)^2 + \left(\frac{4u_r}{r}\right)^2 + \left(\frac{u_E}{u}\right)^2 + \left(\frac{u_d}{d}\right)^2}$$

Equation 3

Where $u_F$, $u_l$, $u_r$, $u_E$, and $u_d$ are the uncertainties of the force, beam length, beam radius, Young’s modulous of glass, and the beam deflection, respectively. The values on the right hand side of the equation combine to give an error estimate of about 5% for the force measurement. Stress measurements are assumed to be analogous to force measurements in this case.

Error due to measurements of fiber cross-sectional area is assumed to be negligible because of the large magnification achieved on the SEM.
2.5 Cross-Linking

As a modification to the micromechanical testing experiments, some fibers were treated with 8% glutaraldehyde (a lysine cross-linker) (Zhang et al. 2003). This cross-linking was preformed underwater prior to the dehydration process described above and allowed to react for 30 minutes. Immediately following the 30 minutes, 100% ethanol was used to serially replace the glutaraldehyde in the chamber and dehydrate the fiber. It is assumed that any chemical cross-linking activity stopped at that time.

2.6 Recovery Experiments

Individual fibers were transferred in their native packed form into a dH$_2$O filled chamber (Figure 13 (B)) using a sharpened toothpick. The fibers were allowed sometime to unravel (ca. 10 minutes), then were tied at both ends to vertical stainless steel rods (ca. 0.3 mm). One rod was mounted in a stationary Plexiglas platform using ('3M' 5200 Marine Adhesive Sealant – Fast Cure) and the other was mounted on a movable platform that was adjustable by a micrometer (movement restricted to one dimension). To secure the fibers they were wrapped around the steel rods several times, then carefully tied using square knots.

Thread annealing was accomplished by allowing the fibers to remain stretched at a given strain, and several annealing times were tested for this investigation. Stretching was followed by various annealing treatments:

1. The fibers were allowed to anneal at their maximal strain
2. The fibers were allowed to contract by 20% then held at that new strain
3. The fibers were not allowed to anneal at all and immediately allowed to recover
Recovery was measured on a logarithmic time scale: at 1, 10, 30, 100, 1000, and 10000 minutes. Instantaneous recovery was also measured at 20, 40, and 60 seconds. It proved difficult to operate at times shorter than 20 seconds due to human physical limitations.

Recovery measurements were achieved by noting the initial micrometer position and initial fiber length; stretching the fiber to a given maximal strain, then reading off the micrometer position at the given relaxation times. Fiber strain was calculated by adding the difference in initial and measured micrometer position to the initial fiber length, then dividing by the initial fiber length.

2.6.1 Varying the Relative Humidity

A series of recovery tests was performed at 97% constant relative humidity. Fibers were first isolated and mounded as above, but were then dehydrated as per micromechanical tests. Following dehydration, the fibers were sealed (airtight) in their chamber in the presence of a saturated potassium sulfate solution (salt in excess) at 20 °C (Merck, 1976) and were allowed to recover for a month.

2.6.2 Cross-Linking effects on recovery

As a modification to the recovery testing experiments some fibers were treated with 8% glutaraldehyde (a lysine cross linker). This cross-linking was preformed underwater and allowed to react for 30 minutes. Cross-linked fibers were not allowed annealing time subsequently to the cross-linking time, and only fibers held at their maximal strain were tested. It is assumed little annealing took place during the cross-linking procedures.
2.6.3 Uncertainty of Recovery Measurements

In order to allow the fiber the possibility of recovery, it had to be left completely relaxed for long periods of time. When the time to make a measurement came, the fiber had to be extended to the point of just taut; this was the major variable as the decision was relatively subjective. In order to quantify this error, independent measurements of unstretched fibers were also undertaken, and the resulting range of lengths was used as the uncertainty of all fiber length measurements.

2.7 Birefringence

** This method is not presented in the results section as it was developed as a means to quantitatively measure structural change due to processing. Nevertheless it is a result of this thesis. **

Birefringence (B) is the numerical difference between the two indices of light refraction of an anisotropic material, where $n_0$ is the refraction of the ordinary ray and $n_e$ is the refraction of the extraordinary ray (Wood, 1964), as described by the following equation:

$$ B = n_e - n_0 $$

Equation 4

Birefringence was calculated in accordance with Kliger et al. (1990) as $\Gamma/d$ where $\Gamma$ is the optical retardation measured using a Senarmont $\lambda/4$ compensator plate (described below) and $d$ is the thread diameter (path length) also measured by optical density techniques (described below). This relationship is described by the following equation:

$$ B = \Gamma/d $$

Equation 5

The birefringence of fibers immersed in distilled water was measured in order to quantify the degree of optical anisotropy in the fiber in response to draw
transformation and annealing. This optical anisotropy will reflect changes in molecular conformation that include the stress induced alignment of unstructured protein chains, as well as the formation or disruption of ordered α-helix or β-sheet domains. Fibers were mounted under a cover glass in a chamber similar to the one used for the recovery experiments. This chamber was substantially shallower as it had to fit onto the stage of a Leitz orthoplan polarizing microscope (Ernst Leitz Canada, Midland, Ontario). A 32x long working distance polarizing lens (NA = 0.4) was used in combination with a Diagnostic Instruments INC. RT™ SE6 Monochrome digital camera (CCD Grade 0 @ 1360x1024 Pixels) and a 546 nm (green) filter to capture a series of images (Figure 14).

**Figure 14.** A typical image of a hagfish fiber captured on a cross polarized dark field of a Leitz orthoplan polarizing microscope.

All images were obtained under identical lighting, aperture, and shutter conditions, and were later analyzed for light intensity across the fiber using
imageJ software (version 1.33u, by Wayne Rasband, National Institutes of Health, USA). All images were later manually corrected for the CCD bias as well as for the base line light that managed to escape the cross polarization (described below). This was achieved by taking a bias picture – shutter speed and aperture identical to all the others, but with a completely closed camera reflex.

### 2.7.1 Intensity Histogram Analysis

Captured dark field intensity images were converted into optical density histograms expressed as relative intensity (Figure 15). The derivation of relative intensity is described below.

![Intensity histogram](image)

**Figure 15.** Intensity histogram for the hagfish fiber image shown above. Relative intensity refers to average brightness perceived, relative to a minimum background calibration (later corrected) for each column of pixels along the x axis. Pixel position simply refers to the position along the x axis of the digital camera's CCD. Insert
demonstrates that the maximum intensity is not an outlier, rather a continuum.

2.7.2 Calibration of Retardation Measurements

The relationship between intensity and the birefringent retardation was found to follow a linear relationship. A Senarmont $\lambda/4$ compensator, in combination with a 546 nm (green) filter, was used in the calibration process according to the supplement instruction manual for a Wild M21 polarizing microscope. Intensity measurements were obtained for a blank field of view at increasing analyzer angles of rotation ($\theta$ in Figure 16) (from $0^\circ$ to $20^\circ$ rotation). The intensity values presented in Figure 17 are plotted against $\sin^2\theta$ values in order to achieve a linear relationship. The relationship described here was used to convert peak intensity values for fibers (e.g. Figure 15) into retardation values. These data are extracted from a series of sequential measurements performed, and this calibration is later used for the determination of the birefringence of a given fiber. The slope (the retardation constant) was found to have the dimensionless magnitude of 2387.3. Figure 16 outlines the steps taken for each intensity image obtained to derive the birefringence value for the specific fiber. For each image the relative intensity ($I_r$) was found using the following equation:

$$I_r = \text{Relative Intensity} = \frac{\text{Average Intensity} - (\text{Empty Field Intensity} - \text{Bias Frame})}{\text{Empty Field Intensity} - \text{Bias Frame}} \quad \text{Equation 6}$$

Where average intensity is the average value of intensity for a given column of pixels; empty field intensity is an average intensity value obtained for that same column of pixels, but when no objects are visible on the dark field; and bias frame was the average intensity for that same column of pixels when no light was available (closed shutter). The method for deriving the slope of the relationship is described below.
Figure 16. Example of derivation of birefringence from a measured intensity level. Angle of rotation ($\theta$) of the analyzer was linearized by finding the $\sin^2$ relationship to relative intensity and the retardation constant, as demonstrated by the inserted formula. Y axis demonstrates a corrected relative intensity. X axis refers to a previous calibration of angle of rotation using the cross-polarizing microscope's analyzer (discussed below). The equation follows the calculation necessary to obtain a birefringence value.

\[
\begin{align*}
I_r &= \frac{I}{I_0} - 1 \\
I_r &= K \sin^2 \theta \\
\Rightarrow \colon \quad \theta &= \arcsin \sqrt{\frac{I_r}{K}} \\
\text{Retardation} &= \theta(3.03 \text{ nm/} \theta) \\
\text{Birefringence} &= \frac{\text{Retardation}}{\text{Fiber Diameter}}
\end{align*}
\]
Figure 17. Sample intensity levels of a blank field were obtained by rotating the polarizing microscope's analyzer through known angles. 546 nm filter and a λ/4 plate were also used. These data were used for the determination of the retardation constant of the birefringence apparatus used. Y axis shows intensity relative to that of 0° analyzer rotation at a maximally cross polarized dark field. X axis shows the sin² value of the angle that the analyzer was rotated to.

2.7.3 Birefringence Diameter Measurements

Birefringence is defined as the ratio of the retardation divided by the optical path length. The path length was determined from fiber diameter as follows. Intensity (optical density) diameter measurements were accomplished by adopting the method developed by Knight and Parsons (1985) for measuring thin cylindrical objects under light microscopy conditions. Line A-B in Figure 18 (insert)
represents the width of the intensity distribution at 2/3 of the height of the distribution. This width can then be expressed as the fiber diameter by comparison to an image of a B&L calibration micrometer slide (5.04 pixels/μm).

![Graph showing relative intensity vs. pixel position](image)

**Figure 18.** Line A-B represents the location and dimensions of the diameter as calculated from the intensity histogram of the hagfish fiber shown above. 5.04 pixels/μm ratio was used for measurements at the 32x magnification.

In support of the above method of optical diameter determination Figure 19 demonstrates the above calculations to be accurate within about 10% by comparing the results to SEM data collected on the same fibers. An average of three light microscopy measurements were collected from light density histograms at 32X magnification and compared to the average of 15 measurements collected from 15000x images collected on the SEM.
Figure 19. Light microscopy diameters closely match SEM diameter measurements. X and Y error bars represent standard error. Intensity Levels are the average of three measurements. SEM diameters are the average of 15 measurements. Light gray line represents the ideal line - a 1:1 relationship between light microscopy and SEM measurements.

2.7.4 Correct Fiber Positioning

Several steps were taken to reduce error due to incorrect fiber positioning. To demonstrate uniformity in the fibers a series of precision measurements are presented below.

Normally all measurements took place at 200 μm from the fiber’s point of attachment (on the stainless steel post). Intra-fiber variation was avoided by not changing the test position from the initial start site. Figure 20 shows the uniformity of the fiber from this normal location of imaging. The data indicate that small errors in fiber positioning would not have lead to any significant differences in final birefringence results. The fiber tested in Figure 20, was
deformed to 45% extension. This specific extension was chosen since a drop in birefringence was always seen at those strains, and therefore the greatest potential variation in data. Nevertheless, very little variation was observed.

![Graph of Fiber Variation](image)

**Figure 20.** Fiber Variation as a function of position at 100\(\mu\)m intervals from the initial start site (200 \(\mu\)m away from attachment point) on a fiber deformed to 45% extension. This shows that the unavoidable variation along the fiber at that extension is still relatively insignificant. Error bars represents standard error in the data.

To demonstrate that the apparatus was precise, a series of birefringence values was obtained for a fiber that was held at a particular extension and at a particular location. Figure 21 shows such a series. A fiber was deformed to 45% extension (for the same reason stated above) and held. Birefringence values were obtained at that exact location over the progress of an hour and resulting variation is shown below.

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Figure 21. Birefringence of a specific location (without varying the location) on a fiber deformed to 45% extension and held for an hour. Note the small variation in birefringence over time – demonstrating a high degree of precision. Error bars represent standard error in the data.

To demonstrate the uniformity of the fiber and the insignificance of a potential mistake by moving the location of testing along the fiber, a series of birefringence measurements, 1 mm apart, were taken. This magnitude of movement would have never occurred during an experiment, but the data is shown in support of the uniformity of the fiber. Figure 22 shows the little variation in birefringence seen along the entire length of a fiber mounted in the above birefringence testing chamber.
**Figure 22.** Birefringence of a fiber deformed to 45% extension measured at 1mm intervals away from initial start site. Note the small variation in birefringence along the entire length of the fiber – suggesting great uniformity. Error bars represent standard error in the data.
3 RESULTS

3.1 Recovery

The ability of the fibers to recover to their native state was quantified by measuring the change in a fiber’s resting length over time. Recovery as a function of strain, time and annealing time was measured. This behaviour is an important factor in trying to design a stable material – one that remains processed and does not return to some native state.

3.1.1 Instantaneous Recovery

Figure 23 shows that fibers deformed to either 150% or 80% extensions exhibited an immediate recovery of up to about 20% of their initial length (150% or 80% respectively). Temporally, this recovery was rapid - it lasted in the order of a few seconds and proved difficult to quantify due to human limitations. The fibers did not exhibit any further significant recovery during the first few minutes of the experiment in support of Fudge et al.’s observations (2003) (Figure 8). Strain was normalized by expressing the ratio of resting fiber length at the tested recovery time versus its length at the beginning of the experiment (either 150% or 80% extensions). These data clearly demonstrate that recovery starts immediately upon release of tension from the fiber. The data also suggest that these fibers may continue to recover if allowed to. Moreover, these data provide evidence for the amorphous terminal domain of Fudge et al.’s model. The domains are amorphous and are, therefore, able to spring back to their native disordered state upon removal of tension from the fiber.
Figure 23. Instantaneous recovery upon removal of stress in fibers taken to 80% or 150% extension and held at that extension for one minute. Normalized strain represents the ratio between post- to pre-recovery strains. Error bars represent the standard error among the data.

3.1.2 Long Term Recovery

When deformed fibers were allowed to recover for long periods of time, fibers that were held for 5 minutes or less recovered most of the deformation. The fibers in Figure 24 were held at their maximum deformed length for 1 or 5 minutes and similarly to above, standardized strain refers to the ratio of post-strain to pre-strain of the fibers, except all these fibers were deformed to 150% extension. The data strongly suggest that contrary to previous belief, the fibers were not dimensionally stable. In fact they appear to return to their native length after about 5 days at room temperature. This recovery suggests that whatever β-sheets formed at 150% extension were not stable and over time unfolded and returned to their initial α-helical conformation. Fudge et al.'s (2003) final recovered standardized strain is shown as a single point in the figure.
Figure 24. Allowing the fibers to recover for a longer period of time uncovers a hidden tendency to resort back to their native form. These fibers were all initially deformed to 150% extension then held for the time indicated. Fudge et al.'s (2003) final recovered standardized strain is shown as a single point. Error bars represent standard error in the data.

3.1.3 Annealing

Figure 25 shows how physical stability was achieved by holding the fibers for longer time periods at their maximum deformed state. This treatment potentially allowed the newly formed β-sheets enough time to interact with each other (Anneal) and convey this increased dimensional stability. Fibers that were held at a strain for 30 minutes or longer at most only recovered the same initial,
normally instantaneous, 20% of the total maximum deformity seen in Figure 23. This was probably a result of the amorphous domains of the dimers not stabilizing during the time allotted for annealing (insufficient hydrogen bonding). Even fibers held for very long periods of time (2000 minutes \(\approx 1.4\) days) recovered some of the maximum deformity. This may mean that complete dimensional stability will never be achieved via physical interactions alone – either the environment must change, or some chemical intervention may be required.

Figure 25. Long term recovery in deformed fibers allowed annealing for various times. These fibers were all deformed to 150% extension then held for the time indicated. Standardized strain refers to the ratio of post-strain to pre-strain. Fudge et al.’s (2003) final recovered standardized strain is shown as a single point. Error bars represent standard error in the data. Dashed data represents the 1 and 5 minutes held fibers from the previous figure.
3.1.4 Relative Humidity

Throughout the experiments water was used as the plasticizer - all recovery experiments were done underwater. Figure 26 demonstrates that a small (3%) reduction in the availability of a plasticizer would have a significant effect on the recovery behaviour of the fibers. Fibers were held for 1 minute at a maximum strain of 150% and then allowed to recover in 97% relative humidity. Although no other manipulation was done, and although when tested underwater the fibers completely recovered, no recovery was evident at a lower relative humidity level.

![Graph](image)

**Figure 26.** A small (3%) reduction in the environmental water content yields a major functional variation in the temporal stability of the fibers. These fibers were deformed to 150% extension and upon placement in 97% relative humidity allowed to recover. Note the scales on the Y and X axis – fibers recovered about 10% over the much longer recovery time period (a month). Error bars represent standard error in the data.

These data illustrate the importance of water in the system. Dropping the relative humidity only 3% prevented the normal recovery pattern seen above. After more
than a month of allowed recovery time, no recovery was detected. Following the trial period, the fibers were immersed in water again and proceeded to behave as before – returning to their native state over a five day period. From a material engineering point of view this is bad. A simple change in the environmental conditions (e.g. material gets wet) can lead to catastrophic instability. Therefore further manipulation was required to make the fibers immune to environmental changes, and thus truly dimensionally stable.

3.1.5 Cross-Linking

In order to achieve dimensionally stable fibers, cross-linking of the numerous lysine R groups present in the intermediate filament based hagfish fibers (Koch et al. 1995) was achieved using a common protein covalent cross-linker – glutaraldehyde (Zhang et al. 2003). Figure 27 shows that this chemical stabilization achieved what the annealing alone could not – dimensional stability of the complete fiber, including its dimers' amorphous terminal domains. Cross-linking fibers at some maximal deformation (150% extension) prevented any significant recovery (less than 0.5%) thus providing a system to create a dimensionally stable material that will retain its characteristics regardless of the time scale, or relative humidity of its environment.
Figure 27. Chemical stabilization of the fibers works to prevent any significant recovery. These fibers were deformed to 150% extension before cross-linked using gluteraldehyde for 30 minutes. Note the scale on the Y axis — changes in strain were below 0.5% and any deviations from the original strain were probably due to measurement error. Error bars represent standard error in the data.

3.1.6 Discussion — Recovery

Although on a very different time scale, fibers seemed to follow the pattern of wool recovery suggested by Hearle (2000). This does not contradict Fudge et al.'s (2003) observations of irreversible recovery; rather it adds on a temporal level of complexity. Hagfish fibers are not embedded in a matrix like that of wool, and since the interactions between the individual intermediate filaments and the matrix are still in question (such as potentially preventing proper unfolding of the coiled-coil domains, or fiber-to-fiber interactions), it is possible that the much expanded recovery time scale is simply due to partial physical stabilization (annealing) that occurs in the fibers upon deformation and/or because of the elasticity of the wool matrix. This process of annealing may simply allow the fibers more time to form extensive hydrogen bonds not only among the newly formed β-sheets - both on the intra- and inter-dimer (coiled-coil) levels, but also
among individual intermediate filaments that may draw nearer to each other upon deformation (extension).

Annealing alone did not seem to completely stabilize the fiber – as seen by the nearly immediate recoil of the newly released fiber. This recoil may be the result of the amorphous IF terminal domains returning to their higher entropic state, and suggests that hydrogen bonds alone are insufficient for their fixation. However, this recoil problem was solved by covalently bonding the amorphous termini in their extended state using a chemical cross-linker.

It became evident that environmental conditions play a major role in the behaviour of the fibers. Changing the humidity level alone, ever so slightly, resulted in a major recovery behaviour change. Fibers appeared to stabilize with no need for any processing, but this stabilization was false, as the fibers retorted to their native state when the environment changed back.

### 3.2 Birefringence

#### 3.2.1 Birefringence of a Deformed Fiber

Spatial deformation increases axial alignment of hagfish fibers. In accordance with preliminary observations made by Fudge et al. (2002), as hagfish fibers are extended their birefringence increases. The data in Figure 28 demonstrate the draw processing effect on the molecular alignment within the fibers. Alignment, and increases from 0% to 25% extension as the normally amorphous, terminal domains extend (and orient), but it drops at ca. 50% extension, once the α-helical coiled-coil domains start unraveling. This drop in birefringence is soon recovered as deformation continues, likely because β-sheets are forming. However, this rise in birefringence could also arise from the elongation and alignment of disordered protein chains formed when the α-helices are disrupted.
Figure 28. Typical representation of the effect of axial deformation on crystallization in hagfish fibers. Note the drop in birefringence after the 25% extension due to the conformational change in the α-helices that form the coiled-coil domains of the dimers. These get disrupted as the fiber passes its yield point at about 35% extension.

3.2.2 Birefringence of an Annealed Fiber

Annealing allows for an increase in crystallization – this is a potential method for partial fiber stabilization. Figure 29 shows birefringence values collected for a typical fiber deformed to 150% extension and left slack at 125% extension for 1.5 hours. The data clearly indicate that crystal formation in the fiber, via hydrogen bond formation among the β-sheets, is dependent on the time allowed for this annealing reaction. The longer the annealing reaction is allowed to run, the higher the birefringence; and therefore the higher the degree of β-sheet crystal formation. This is the first real substantial direct evidence that β-sheet crystals do form, rather than simply oriented amorphous intermediate filaments terminal domains (due to the draw processing) – the only way for birefringence to increase in a fiber that is held at a fixed length is if there is an increase in crystal formation. This also strongly supports the recovery observations made above and
suggests that a fiber held for 2000 minutes should be highly birefringent and should have an extensive $\beta$-sheet crystal network.

![Graph showing birefringence over annealing time](image)

**Figure 29.** Typical representation of the effect of annealing on the degree of crystallization. This fiber was deformed to a 150% extension and brought back to 125% before being allowed to anneal. Note how birefringence increases over time – roughly a 50% increase in birefringence over 90 minutes – suggesting increased crystal formation as the annealing reaction proceeds.

**Birefringence of a Cross-Linked Fiber**

To determine if annealing can occur after chemical cross-linking, data collected showed no significant increase in the degree of crystal formation as a result of cross-linking. Figure 30 shows the lack of change in birefringence values obtained for a typical fiber deformed to 100% extension and immediately cross-linked. 100% was used instead of 150% so as to prevent reaching some ceiling of birefringence – in case birefringence increased substantially. Fibers were allowed to form covalent bonds for 90 minutes – although the same time period showed significant increases in birefringence due to annealing in uncross-linked fibers.
little to no crystalline formation occurred in these fibers. These data indicate that cross-linking (chemical fixation) also prevents annealing of the β-sheets, probably via reduction of intra-fiber motility – adding another degree of fine-tuning control onto this system.

![Graph](image)

**Figure 30.** A typical experiment demonstrating cross-linking’s inhibitory effects on physical stabilization of the fiber - no increase in birefringence is visible over the tested time period. Fibers were deformed to a 100% strain in order to avoid possible ceiling of the data (avoid a possible maximum).

### 3.2.3 Discussion – Birefringence

Birefringence seems to provide a quick, simple method for quantifying the structural change in a deformed fiber. This method seems to be highly replicable, precise, and relatively accurate. Some more direct support from established methods (X ray diffraction) is probably still required to formally cement this approach, but the data are promising and seem to be consistent with results
published by Fudge et al. (2003). The results from the annealing experiment are very exciting. These data clearly and directly demonstrate an increase in birefringence in an annealed fiber. Since the fiber is held at a fixed length, the only way for birefringence to increase is an actual increase in crystallinity. Interestingly enough cross-linking prevents annealing, this is insightful and probably means that the covalent bonds restrict free movement on the intra-intermediate filament level, thus limiting the ability to fully form proper β-sheets. No recovery is seen in these fibers probably because of the extent to which the fibers are covalently cross-linked.

### 3.3 Micromechanical Testing

As a result of the above recovery and birefringence methods it became evident that in order to produce a fiber that exhibits both superior mechanical properties and dimensional stability two criteria must be satisfied. 1. Assuming β-sheet crystals convey increased performance (Fudge et al. 2003), the fibers must be deformed to extensions nearing 150%. Since fibers exhibit long term recovery, these fibers must also be physically or chemically stabilized. 2. As shown above, physical stabilization was insufficient in achieving true environmental stability. Therefore chemical covalent cross-linking must also be preformed, and the fibers must be tested at varying levels of plasticizer availability.
3.3.1 X-Linking Affects Mechanics

Figure 31 shows the dramatic changes that occur following chemical cross-linking of an otherwise unprocessed fiber. Extensibility of the cross-linked fiber (A) decreased and strength increased compared to completely unprocessed fibers (B) (Fudge et al., 2003). Toughness increased to 170 MJ/m³ and Initial Stiffness ($E_{int}$) increased 400 fold to 2 GPa. The covalent cross-links seem to fix everything in place, preventing much extensibility even if the terminal domains are unstrained. This explains the 400 fold increase in the initial modulus – since it is covalent bonds that now get loaded by force instead of much weaker hydrogen bonds, the fiber can immediately bear a greater load. These changes are presented in Table 4 below.

![Graph A showing stress-strain relationship](image1.jpg)

**Figure 31.** Cross-linking (A) resulted in loss of extensibility, but a marked increase is seen in stress tolerance (at least double that of an uncross-linked fiber (B)), toughness and initial modulus (values given in Table 4). Note that axis scales are different in A and B. Figure B is from Fudge et al. (2003).
Table 4. Average tensile mechanical properties of cross-linked hagfish fibers compared to unprocessed hagfish fibers (Fudge et al. 2003). Note the 400 fold increase in initial stiffness and the increase in toughness. Without further manipulation this cross-linked fiber easily rivals spider dragline silk (Gosline et al., 1999).

<table>
<thead>
<tr>
<th>Material</th>
<th>Stiffness $E_{int}$ (GPa)</th>
<th>Strength at Break $\sigma_{max}$ (GPa)</th>
<th>Extensibility $E_{max}$ $(\Delta L/L)$</th>
<th>Toughness (MJ/m$^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unprocessed, wet</td>
<td>0.005</td>
<td>0.2</td>
<td>2.5</td>
<td>130</td>
</tr>
<tr>
<td>Cross-linked, wet</td>
<td>2</td>
<td>0.5</td>
<td>0.7</td>
<td>170</td>
</tr>
<tr>
<td>&lt;spider dragline silk&gt;</td>
<td>10</td>
<td>1.2</td>
<td>0.3</td>
<td>160</td>
</tr>
</tbody>
</table>

3.3.2 Plasticizer is Important, But Not Critical

Figure 32 shows that in the absence of other processing, cross-linked fibers remain relatively stable whether hydrated or not. Water still acts as a plasticizer, allowing the fibers to deform to a much greater extent before failure (A). However, in the absence of water fibers surprisingly withstand greater stresses (0.5 GPa when wet; 0.8 GPa when dry), and as expected display higher initial stiffness (2 GPa when wet; 3.6 GPa when dry) (B). Table 5 presents average values for these variables alongside those for unprocessed (shown above) and spider dragline silk (Gosline et al. 1999). Comparing these new properties to spider dragline silk demonstrates the effectiveness of cross-linking alone. These fibers are now tougher than spider dragline silk (hagfish: 200 MJ/m$^3$; spider: 160 MJ/m$^3$). However spider dragline silk can still withstand higher stresses (hagfish: 0.8 GPa; spider: 1.2 GPa) and is still much stiffer (hagfish: 3.6 GPa; spider: 10 GPa). Therefore hagfish fibers require further processing to achieve comparable tensile mechanical properties to those of spider dragline silk.
Figure 32. Tensile mechanical properties of undeformed (0% extension) cross-linked fibers in the presence of a plasticizer (A) versus its absence (B). Data for both are plotted under the same axis scales. Note the higher initial stiffness and final failure stress of the dry fibers (Values are presented in Table 5).

Table 5. Average tensile mechanical properties of 0% extended and cross-linked hagfish fibers. Note the increase in toughness in the dry fibers. Unprocessed hagfish fibers and spider dragline silk data are presented as a reference (Fudge et al., 2003; Gosline et al., 1999 respectively).

<table>
<thead>
<tr>
<th>Material</th>
<th>Stiffness E_{int} (GPa)</th>
<th>Strength at Break σ_{max} (GPa)</th>
<th>Extensibility E_{max} (ΔL/L)</th>
<th>Toughness (MJ/m³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unprocessed fibers</td>
<td>0.005</td>
<td>0.2</td>
<td>2.5</td>
<td>130</td>
</tr>
<tr>
<td>Cross-linked, 0% extension, wet</td>
<td>2</td>
<td>0.5</td>
<td>0.7</td>
<td>170</td>
</tr>
<tr>
<td>Cross-linked, 0% extension, dry</td>
<td>3.6</td>
<td>0.8</td>
<td>0.45</td>
<td>200</td>
</tr>
<tr>
<td>&lt;Spider dragline silk&gt;</td>
<td>10</td>
<td>1.2</td>
<td>0.3</td>
<td>160</td>
</tr>
</tbody>
</table>
3.3.3 Draw Processing leads to surprising results

Figure 33 shows that as expected deforming the fibers to a 150% extension does significantly increase the initial stiffness. However, a reduction in the toughness of the fiber is also exhibited (Table 6). Again, removal of plasticizer does affect the extensibility and initial stiffness. Wet fibers (A) are more extensible than dry (B), but far more impressive is the significant increase in initial stiffness of the dry fibers (B) to a stiffness of about 10 GPa. Table 6 shows the average tensile values for the two treatments.

![Graph A](image.png)

![Graph B](image.png)

**Figure 33.** Tensile mechanical properties of fibers deformed to a strain of 150% extension and cross-linked. Both wet (A) and dry (B) treatments display increased stiffness, but decreased toughness compared to those not deformed (Figure 32). Axis scale for both figures is identical. Note the much higher stiffness of the dry fibers (B). Value are presented in the table below.
Table 6. Average tensile mechanical properties of 150% extended and cross-linked hagfish fibers. Note the increased initial stiffness in the dry fibers. Spider dragline silk data is presented as a reference (Gosline et al. 1999).

<table>
<thead>
<tr>
<th>Material</th>
<th>Stiffness $E_{\text{int}}$ (GPa)</th>
<th>Strength at Break $\sigma_{\text{max}}$ (GPa)</th>
<th>Extensibility $E_{\text{max}}$ (ΔL/L)</th>
<th>Toughness (MJ/m³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross-linked, 150% extension, wet</td>
<td>1.5</td>
<td>0.85</td>
<td>0.25</td>
<td>100</td>
</tr>
<tr>
<td>Cross-linked, 150% extension, dry</td>
<td>10</td>
<td>0.75</td>
<td>0.15</td>
<td>60</td>
</tr>
<tr>
<td>&lt;Spider dragline silk&gt;</td>
<td>10</td>
<td>1.2</td>
<td>0.3</td>
<td>160</td>
</tr>
</tbody>
</table>

3.3.4 A Surprising Terminal Domain Twist

Figure 34 shows an interesting result achieved from applying a small deformation to the fibers – to 20% extension. This should be sufficient in stretching the amorphous terminal domains of the dimers without placing any substantial stress on the coiled-coils domains. Since the fibers are cross-linked – it makes sense that the system is very stiff, thus displaying a high initial modulus (Table 7). However, the fibers behave almost identically to those deformed to 150% extension, suggesting that the covalent bonds resulting from the cross-linking dominate the system regardless of other processing. Again the dry fibers display a much higher initial stiffness (B). Table 7 shows the average tensile mechanical properties for the two treatments.
Figure 34. These fibers were deformed to 20% extension before being cross-linked – just enough to strain the terminal domains, but not the coiled-coils. Wet (A) and dry (B) resulted in very similar mechanical properties (table below).

Table 7. Average tensile mechanical properties of 20% extended and cross-linked hagfish fibers. Note how similar the values are to those presented in Table 6. Spider dragline silk data is presented as a reference (Gosline et al. 1999).

<table>
<thead>
<tr>
<th>Material</th>
<th>Stiffness $E_{\text{int}}$ (GPa)</th>
<th>Strength at Break $\sigma_{\text{max}}$ (GPa)</th>
<th>Extensibility $E_{\text{max}}$ ($\Delta L/L$)</th>
<th>Toughness (MJ/m$^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross-linked, 20% extension, wet</td>
<td>1.8</td>
<td>0.6</td>
<td>0.25</td>
<td>80</td>
</tr>
<tr>
<td>Cross-linked, 20% extension, dry</td>
<td>8.5</td>
<td>0.75</td>
<td>0.15</td>
<td>80</td>
</tr>
<tr>
<td>&lt;Spider dragline silk&gt;</td>
<td>10</td>
<td>1.2</td>
<td>0.3</td>
<td>160</td>
</tr>
</tbody>
</table>
3.3.5 Taking Advantage of the Terminal Domains

Figure 35 shows data obtained for fibers that were deformed to 150% extension, but slackened back to 130% before cross-linking occurred. The intention was to gain back the extra extensibility exhibited by fibers that were not deformed (Figure 32) by allowing the terminal domains to return to their native disordered (amorphous) state. However, not all the extensibility was regained probably due to the tension the fiber was under at the 130%. It is quite likely that because the β-sheets are not immediately stabilized (as seen above) when the fibers were slackened off - instead of only the amorphous terminal domains recoiling, some of the coiled-coils also recoiled a bit. Wet fibers (A) exhibit a much higher (220 MJ/m³) toughness compared to the dry fibers (B) (50 MJ/m³); whereas dry fibers exhibit a much higher initial stiffness. Table 8 shows a summery of the average tensile mechanical properties for these two treatments.

Figure 35. These fibers were deformed to 150% extension, then slackened back to 130% extension before cross-linked. Both wet (A) and dry (B) treatments show substantial increase in extensibility and breaking yield stress. Note the increased Stress scale compared to previous figures. Average tensile mechanical values are presented in the table below.
Table 8. Average tensile mechanical properties of hagfish fibers deformed to 150% extension then slackened back to 130% extension before cross-linked. Note the impressive tensile properties displayed by the wet fibers. Spider dragline silk data is presented as a reference (Gosline et al. 1999).

<table>
<thead>
<tr>
<th>Material</th>
<th>Stiffness $E_{\text{int}}$ (GPa)</th>
<th>Strength at Break $\sigma_{\text{max}}$ (GPa)</th>
<th>Extensibility $E_{\text{max}}$ ($\Delta L/L$)</th>
<th>Toughness (MJ/m$^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross-linked, 150% to 130% extension, wet</td>
<td>2</td>
<td>1.1</td>
<td>0.4</td>
<td>220</td>
</tr>
<tr>
<td>Cross-linked, 150% to 130% extension, dry</td>
<td>10</td>
<td>1</td>
<td>0.1</td>
<td>50</td>
</tr>
<tr>
<td>&lt;Spider dragline silk&gt;</td>
<td>10</td>
<td>1.2</td>
<td>0.3</td>
<td>160</td>
</tr>
</tbody>
</table>

3.3.6 Discussion – Micromechanical Testing

The above micromechanical results could easily be mistaken for the spider dragline silk data presented by Gosline et al. (1999). It is impressive that an $\alpha$-helix based system can behave just like a $\beta$-sheet system – there must be extensive covalent cross-linking occurring to explain the high-performance properties displayed by some of the above processed fibers. Together with the stability of recovery achieved via annealing and cross-linking these mechanical data promise a new super material that is not only environmentally friendly, but also tunable to a plethora of dimensional properties.
The data presented in this thesis supports the hagfish dimer model postulated by Fudge et al. (2003). Although dimensional stability differences are seen (recovery), it is likely that these arise from temporal trials not tested by Fudge et al. (2003). The recovery seen explains in part why hagfish fibers seem to behave differently from keratin (Hearle, 2000), as noted by Fudge et al. (2003). The birefringence data presented suggests that β-sheets are formed upon deformation of the fibers past 35% extension, but this thesis demonstrates that β-sheet crystals only form if the fibers are allowed to anneal. This supports the first hypothesis made that the reversible α-helix ↔ β-sheet transition, although a reversible one, can be made permanent via physical and/or chemical manipulations.

The data suggest that keratin should also show irreversibility if β-sheets formed will also be allowed to anneal. This may prove difficult to demonstrate, as high temperatures may be required to strain the keratin well past its yield point in order to achieve extensive α-helix → β-sheet transition. Another hurdle faced may arise from the protein matrix that embeds the intermediate filaments (IF) that form the keratin. This matrix may inhibit IF-to-IF hydrogen bonding and therefore any extensive β-sheet crystal formation simply by preventing the IF from drawing near to one another. In addition, the elastic recoil of the rubber-like keratin matrix (Hearle, 2000) may destabilize any β-sheet structures that form.

Schwaiger et al. (2002) test single coiled-coil domains of rabbit myosin using atomic force microscopy (AFM) (Figure 36). This molecule is a relative of the IF coiled-coil domain and clearly demonstrates the propensity of the coiled-coil region to undergo α-helix ↔ β-sheet reversibility. Moreover, this molecule exhibits tensile mechanical properties similar to those associated with hagfish fibers. If the data from the single coiled-coil myosin molecule are scaled to macroscopic dimensions, the yield strain is ca. 0.3 (based on an L₀ of ~100 nm) and the yield stress is ca. 25 MPa (Figure 36). These values are based on a cross-
sectional area of $A_0 = 1 \times 10^{-18} \text{ m}^2$ that was derived from the 9.8 Å equatorial spot on the X-ray diffraction pattern presented in Figure 12 (I).

The single myosin coiled-coils tested by Schwaiger et al. (2002) seem to reform rapidly in load-unload cycles lasting 2-10 seconds, in contrast to the recovery time of hagfish fibers. However, hagfish fibers are made up of many bundles of...

**Figure 36.** A single myosin coiled-coil domain (Schwaiger et al. 2002) behaves similarly to intermediate filament-based materials, such as keratin and hagfish fibers. Note the derived stress scale on the right hand side Y-axis (assuming $A_0 = 1 \times 10^{-18} \text{ m}^2$) and the yield stress estimate (25 MPa).
these coiled-coils, and this may provide the opportunity for adjacent molecules to interact to form partial β-sheet crystals that take days rather than seconds to reform. When these interactions are allowed to continue (annealing) they render the α → β transition irreversible. It is likely that this unique property arises from the hierarchical organization of hagfish slime fibers. It is also interesting to postulate what might happen if the single myosin coiled-coils are allowed to anneal. It is possible that these coiled-coils will also form somewhat stable β-sheet crystals. What might be lacking for proper stabilization is the interaction of the coiled-coils with adjacent ones – similarly to the problem experienced by keratin as a byproduct of its IF being embedded in a protein matrix. Thus, it is possible that myosin molecules packed in a myosin fibril might exhibit an irreversible α → β transition similar to that of IF-based materials.

It is puzzling that an α-helical cross-linked fiber that is otherwise unprocessed (Figure 31 (A)) could display almost equivalent tensile mechanical properties to those of a draw transformed, stabilized and chemically cross-linked fiber (Figure 35 (A)). It seems ludicrous that an unprocessed fiber exhibits yield stress of 0.005 GPa and initial stiffness of 0.005 GPa, and that cross-linking alone (without draw transformation, and therefore without any α-helix → β-sheet transition) results in a yield stress of 0.2 GPa (a 40 fold increase) and an initial stiffness of 2 GPa (a 400 fold increase). Could it be that the specific covalent cross-linking locations will serve to explain this phenomenon?

Analysis of the specific amino acid composition of the intermediate filament dimer (Koch et al., 1995), in search of lysine residues that would engage the glutaraldehyde cross-linker used, appears to shed some light on this mystery. The dimers are made up of α and γ chains. The coiled-coil portion of the α chain has 18 lysine groups, and the γ has 17. Of these, 10 lysine residues are lined up enabling easy intra-dimer covalent cross-links. These cross-links could explain the 40 fold rise in yield stress seen (Figure 31). The remaining 8 and 7 (on the α and γ chains respectively) are free to potentially form inter-dimer covalent bonds. This may serve to circumvent the terminal domains, forming a sort of staggered bridge of covalent cross-links (Figure 37) that could prevent the sliding seen in
Figure 9, and may explain the 400 fold increase in initial stiffness seen (Figure 31) via inhibition of the serial force transfer among the amorphous terminal domains discussed above.

![Covalent Bonds](image)

**Figure 37.** Covalent bonds (from cross-linking) may serve to circumvent the elastic behaviour of the terminal domains, inhibiting the sliding of the coiled-coil domains seen in Figure 9.

Interestingly enough, the terminal domain amino acid sequences (Koch et al., 1995) contain very few lysine residues and therefore possess little potential for glutaraldehyde cross-linking. In the N-terminus amorphous domain, the \( \alpha \) chain has one lysine as does the \( \gamma \) chain; and in the C-terminus, the \( \alpha \) chain has one lysine, while the \( \gamma \) chain has two. These should be insufficient to stabilize the terminal domains to the extent the mechanical properties suggest (Figure 31 (A)). This also supports this covalent bridging idea presented above.

Stabilization of the \( \alpha \)-helix \( \leftrightarrow \beta \)-sheet structures formed either via physical or chemical means did provide evidence to support the second hypothesis made. Structural change in the fibers had a direct influence on the fibers' material properties. It is now also easy to imagine a process that will produce high-performance materials made from isolated individual myosin coiled-coils. Hagfish fibers yield at a lower stress than myosin coiled-coils (hagfish: 5 MPa; Myosin: 25 MPa), possibly because of the serial force transfer in their amorphous terminal domains. It is feasible that myosin coiled-coil-based materials will exhibit even superior properties to those displayed by hagfish fibers in this thesis.

Hagfish fibers displayed a plethora of characteristics achieved by fine-tuning the processing procedures. This essentially gives rise to a new high-performance
material. This material displays superior, dimensionally stable tensile mechanical properties that can be adjusted at will. Because of its inherent self-assembly properties and its environmentally friendly building blocks it may prove desirable for many commercial applications.

“I loved deadlines – I love the swooshing sound they make as they pass by...”

- Douglas Adams
Literature Cited


