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COVER SHEET

FINAL (3rd Year) Report to AFOSR on:

BIOMECHANICS OF SPIDER SILKS

Fritz Vollrath, Oxford University, England

Subject F49620-03-1-0111

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BIOMECHANICS OF SPIDER SILKS
Fritz Vollrath, 3rd Year Report (starting date of project May 2003)

SUMMARY AND OVERALL OBJECTIVES OF THE FULL PROJECT

This project aims to gain a fuller understanding of the relationship between processing, structure and mechanical properties of selected spider and silkworm silks. This goal will be pursued by inducing spiders and silkworms to produce threads under a range of controlled processing conditions. The mechanical properties of these fibres will be determined by analysing (again under highly controlled conditions e.g. temperature, humidity) the mechanical behaviour of single filaments under simple stress-strain regimes as well as for cyclic deformation, creep and stress relaxation. These experiments will provide data on the stress-strain relationships of the whole fibre. Some individual components of the fibre fine-structure under loading will be further investigated using a combination of micro-Raman spectroscopy and x-ray diffraction. The mechanical data combined with data on fibre structure will serve to develop and test a battery of specific models for the silks under examination. These models must account for the relative importance of the different hierarchical structural levels for the mechanical properties of silks, to-date no such models exist. Initially these models will be encompassing, as the silks we are studying differ in molecular structure as well as production system. The results of this study will be invaluable not only for understanding native silks but also for the design and spinning (be it biomimetic or traditional) of improved synthetic silk-analogue polymers.
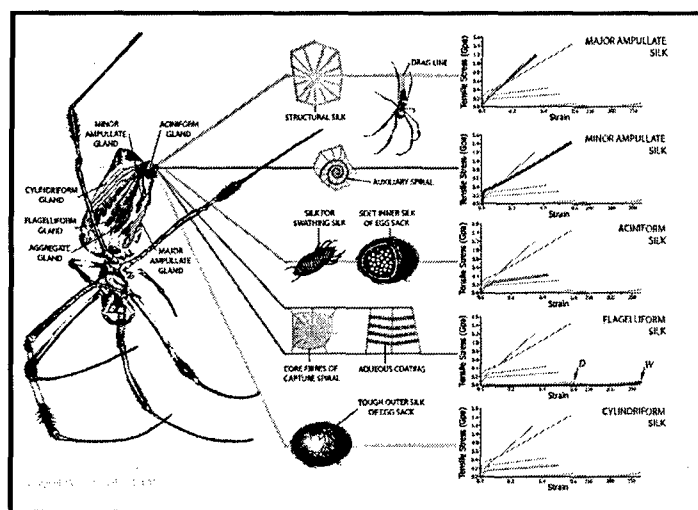
Status of effort: The funds for the three (3) year project were provisionally granted in Feb. 2003, the negotiations between the principal organisations were concluded March 2003 - memo from AFOSR to OxUni dated 18/03/2003 and the first tranche paid out in April 2003 allowing the project to start fully in May 2003.

Accomplishments/New Findings over the 3yr period:

Why look specifically at spider silk? Although silkworm silk is strong, the oscillatory motion of the worm during spinning leads to great fluctuations in mechanical properties. Spider silks, on the other hand, are evenly spun, which provides a model natural fibre with superb combinations of strength and toughness. Such silks range from drag-line with a modulus and strength of about 10GPa and 1GPa respectively to capture thread with a more modest strength, but because of its high extensibility a comparably high toughness.. Spider silk is a strong and tough polypeptide that is optimised by nature to fulfil a wide range of functions using subtle changes in chemical composition and, importantly, morphological structure at a nanometer scale. Attempts to emulate the attractive properties of silk and other natural polymers are frustrated by a lack of quantitative structure-property relations; to redress this deficiency is one of the primary subjects of this grant. To this effect we firstly collected data on the mechanical properties of a wide variety of silks and secondly analysed these data with the view of integrating the information into a comprehensive model. The consistency of the model with the data was the test of our understanding, and this test was to include data not used for the construction of the model as well as data collected later using a wider range of experimental conditions. We were successful, as the publications list testifies, in our attempt to understand silks better than was possible ever before.

In order to develop a quantitative model for silk, we took the radical step of looking at silk from the perspective of a user. Silk fibres are produced by the spider to manage mechanical energy for different tasks without breaking. In particular the silk must store elastic energy in order to support the spider's own weight and in the structural framework of a web it must be able to absorb kinetic energy to capture flying insects. We set out to identify the mechanisms at a molecular level that dictate energy storage and dissipation in a polymer and derive straightforward analytical relations for the full range of mechanical properties that are possible in silk. These relations are expressed in terms of a small number of energy-based parameters with a direct fundamental link to chemical composition and morphological order. In this way, we hope to elucidate some of the key design principles in natural polymers. Moreover, this approach can be applied also to much simpler man-made polymers, thereby offering the potential for the design of improved synthetic polymers. Our studies so far have shown that the full range of thermo-mechanical properties of silk fibres can be predicted from mean field theory for polymers in terms of chemical composition and the degree of order in the polymer structure. Thus, we can demonstrate an inherent simplicity at a macromolecular level in the design principles of natural materials. This surprising observation allows in depth comparison of natural with man-made materials.

Spider silk combines strength and extensibility and a wide range of mechanical properties can be achieved with only minute (if any) changes in chemical structure. The Mean Field modelling approach attempts to integrate strain and tensile stress with a range of relevant

energetic and mechanical parameters such as the loss tangent and potential energy of atomic inter-chain bonding as well as the tensile and bulk elastic moduli. The model reveals that the underlying design principle of silks seems to share an inherent and surprising simplicity at the macromolecular level. We conclude that our modelling approach allows in-depth analysis of natural silks as well as a comparison with synthetic fibres.

To get to his conclusion, which is of high relevance for the ultimate success of the Spiderman project, we have focussed on the data collection and analysis of a series of structure-property relations that predict the wide range of mechanical properties in silks in terms of chemical composition and structural morphology. By translating molecular-level processes that control the storage and dissipation of mechanical energy into bulk mechanical properties, we elucidate the crucial design principles in natural polymers. Indeed, we propose that this energy-based approach allows an in-depth analysis of structure-function relationships in a complex biological fibre. This includes not only the prediction of the dynamic mechanical properties but also allow the calculation of post-yield work hardening and the envelope of conditions of failure-to-break. Interestingly, it appears that this analytical approach can be applied also to the comparatively simpler man-made polymers, thereby offering the potential for the future design not only of biomedical bio-polymers but a much wider range of of improved synthetic polymers.

Our own modelling approach is rather different from previous work on silk (e.g. Gosline and co-workers in 1984 and 1994; Termonia 1994; Thiel and Viney 1995; Perez and co-workers 2000), starting not with the molecular composition to explain global as well as local structure i.e. mesoscale linkages and crystallite packing. Instead we take the selectionist's approach and focus on the 'management' (adapted functionality) of the energy imparted by stressing and straining the filament. Thus, we examine the mechanisms of energy dissipation at the molecular level by examining the energy profiles of atomic inter-chain bonding. In a number of manuscripts we present and model developed in a series of steps, ultimately leading to a full understanding not only of the performance of fibres dry in air but also wetted in the biomedical contect of implantation.

In summary we conclude that silk is not only an interesting material for its own sake but that knowledge (and experimental tools) gained from studying silks the way we did can be used to study other biomaterials as well as compare biomaterials with non-biological polymers (see Vollrath & Porter 's 2006 review in Softmatter).

The second objective of this grant was the investigation of silk as a major component in useful composites. Here we also have made good progress, and discovered a number of interesting and important insights. If we want to commercially employ both spider and silkworm fibres we need to understand their performance in composites. For this purpose we focused to (i) understanding the biological function of the silk cocoons (permeability), (ii) testing and characterising silk fibres (mechanical testing), (iii) develop techniques to investigate the silk and its interfaces (deploying polarization microscopy, linear dichroism, acoustic microscopy), (iv) prepare and characterise composites fibres (by embedding silks in both biological and epoxy-based matrices) and, finally, preparing and

characterize natural cocoon composites.. The information gained by combining the information gathered with these different approached is provide the first key elements needed to obtain high performance silk composites.
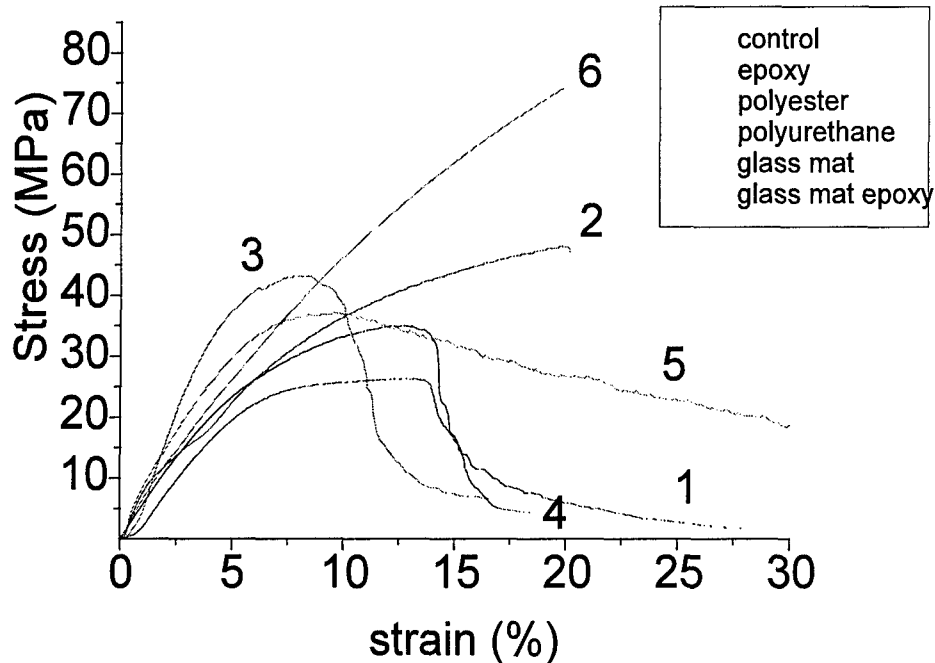
Stress strain curve of silk cocoon composites.

Our work demonstrated that spider (and other silks) can indeed be integrated into composite materials but that both the treatment of the silks and the matrix materials must be carefully chosen and the infusion carefully monitored. As this work will be the focus of a follow-up grant, I here present a summary of our results tabulated in the Appendices 1 a and b. The Key results were the following: after matrix (epoxy and polyester) impregnation the silks and cocoon 'mats' become transparent with the thin cocoon layer composites showing lower stress to break and modulus but improved breaking strain over the native silks. Epoxy composites gave the best mechanical properties while control cocoons treated in acetic acid or ethanol showed similar properties to control cocoon but with less variability within sample. Cocoon silks treated in acetic acid or ethanol showed after epoxy matrix impregnation comparable breaking stress and strain to untreated cocoon composites, but dramatically improved (by a factor of 2) Young's modulus. In general with all matrices and thick cocoon we observed attachment to the sericin coat (sem picture above) and slippage of the silk fibroin fibres. Hence it appears that choosing silk cocoon thin layer and acetic or ethanol treatment will improve both breaking strain and initial modulus. Finally, spider and un-degummed Bombyx silks showed the least response to matrix coating while degummed fibres followed by oven cure showed improved mechanical properties

Personnel: Mr Yi Liu, a student of polymer chemistry at Fudan University joined my laboratory in Oxford in May 2003 to begin experiments on silk. As of Oct 2004 Mr Liu is funded (for a 3 yr period) by a Dorothy Hodgkin Award of the British Government and will continue to be working on issues related to this grant. Negotiations for part-time employment of Dr. David Porter as modeller were concluded in spring 2003 and allowed Dr. Porter to start working on the project in July 2003. The principal RA position was filled by Dr. Cedric Dicko, who started work on Sept. 7th 2004. Dr Dicko's work on the grant impressed the selectors to secure him a 3yr Junior Research Fellowship with the Oxford College St Edmunds Hall starting in Jan 06. In March 2004 Dr Alex Sponner joined my lab for 6 months as RA funded principally from other sources but also collaborating (and funded) on aspects of this grant; as of March 2005 he is funded by an EU-ToK grant but remains working part-time on the AFOSR project.

Publications submitted and published during the 3yr period 2003-2006 that acknowledge support under the AFOSR GRANT F49620-03-1-0111:

2006 Dicko C, Kenney J, Vollrath F β-Silks: Enhancing and controlling aggregation. In pp (in press) in eds A Kajava, J Squire, D Parry: *Fibrous Proteins: Amyloids, Prions and β- proteins. Advances in Protein Chemistry* (vol. 72), Elsevier Press

2006 Olivier E, Le Floch A, Vollrath F The self shape-memory effect in spider draglines. *Nature* (in press)

2006 Vollrath F, Porter Spider silk as archetypal protein elastomer. *Softmatter* (in press)

2005 Sapede D, Seydel T, Forsyth T, Koza MM, Schweins R, Vollrath F, Riekel C Nanofibrillar structure and molecular mobility in spider dragline silk. *Macromolecules* 38, 8447-8453

2005 Liu Y, Shao Z, Vollrath F Linking the physical and mechanical properties of spider silk. *Nature Materials* 4, 901-905

2005 Liu Y, Shao Z, Vollrath F Extended wet-spinning can modify spider silk properties. *Chemical Communications* 2005: 2489–2491

2005 Yang Y, Chen X, Zhou P, Shao Z, Porter D, Knight DP, Vollrath F Toughness of spider silk at high and low temperatures. *Advanced Materials* 17, 83-88

2006 F Vollrath F, Porter D Structure-Property Relations for Silk. *Applied Physics A* 82, 205 - 212

2005 Porter D, Vollrath F, Shao Z, Predicting the mechanical properties of spider silk as a model nanostructured polymer. *European. Physical Journal E* 16: 199-206

2005 Vollrath, F, Sponner A 'The route to synthetic silks' pp.245-270 in *Biodegradable and sustainable fibres.* Ed. R.Blackburn, Woodhead publishing Limited, Cambridge, UK

2005 Sponner A, Schlott B, Vollrath F, Unger E, Frank Grosse F, Weisshart K Characterization of the Protein Components of *Nephila clavipes* dragline silk. *Biochemistry* 44: 4727-4736

2004 Huemmerich D, Scheibel T, Vollrath F, Cohen S, Gat U, Ittah S Synthesis and self-assembly of a spider dragline silk protein in insect cells, *Current Biology* 14; 2070-2074

2004 Riekel C, Roessle M, Sapede D, Vollrath F Influence of CO_2 on the micro-structural properties of spider dragline silk: X-ray microdiffraction results. *Naturwissenschaften* 2004:30-33.

2004 Hronsky M, van Beek JD, Williamson PTF, Vollrath F, Meier BH NMR characterization of native liquid spider dragline silk from *Nephila edulis*. *Biomacromol.* 5; 834-839

2004 Vollrath F, Knight DP Biology and technology of silk production (in press) in (eds A. Steinbüchel and Y. Doi) Biotechnology of biopolymers : from synthesis to patents. Wiley-VCH, Heidelberg & New York (reprinted article in Handbook of Biopolymers 2003)

2004 Terry A, Knight DP, David Porter D, Vollrath F pH induced changes in the rheology of silk fibroin solution from the middle division of Bombyx mori Silkworm. Biomacromolecules 5: 268-277

2004 Dicko C, Knight D, Kenney JM, Vollrath F, Structural conformation of spidroin in solution: A synchrotron radiation circular dichroism study. Biomacromol. 5; 758-767

2004 Dicko C, Kenney JM, Vollrath F, Spider silk-protein refolding is controlled by changing pH. Biomacromol. 5;704-710

Interactions: Talks etcetera (a) In spring 2005 Dr. David Porter attended the AFOSR meeting in San Diego in order to present and discuss the progress of our research with the ASFOR and its other grantees (see his report in the Appendix).   Prof. Vollrath as well Dr. Dicko all visited the Polymer Group at Fudan University several times throughout the grant period to conduct experiments and participate in teaching. Prof. F. Vollrath and Dr. Dicko gave a number of invited talks and lectures at national as well as international polymer and biology conferences and meetings.

AFOSR 'internal' collaborations (b) in summer 2003 visits by Dr Kathy Wahl (Naval Research Laboratory Tribology Section, Code 6176) to Oxford and a return visit by Dr Ann Terry to Washington DC. In 2005 Dr Wahl revisited our Oxford Lab to discuss work in progress and plan new experiments. In 2004 and again 2006 Prof's Fritz Vollrath and David Kaplan (Tufts University) discussed a joint project on spider silk and prepared a pilot document and a pre-application to DARPA.

New Discoveries, Inventions, or Patent Disclosures : No Patents. As to new discoveries we still believe that the modelling work (and relevant publications) by Porter and Vollrath will have a major impact on polymer science.

Honours/Awards: Dorothy Hodgkin PhD Fellowship for Yi Liu (3 yrs)
Junior Research Fellowship from St Edwards College Oxford for Cedric Dicko (3 yrs)
European Union Training and Mobility grant for Dr Alex Sponner (2 yrs)

Markings: n/a

Fritz Vollrath, Oxford, MARCH 1st, 2006

Appendix 1 a and b : Tabulated data from our silk composite experiments.

Appendix 2 review article by Vollrath & Porter (in SOFTMATTER, in press) that summarizes much of the work done under the grant

APPENDIX 1a

Table Mechanical test results for integration of silk cocoon 'mats' into composites				
	Max load (N)	Stress (MPa)	Strain (%)	Modulus (MPa)
Control (thick)	35.54 ± 15.90	29.85 ± 4.77	12.59 ± 2.05	510.98 ± 102.68
Control (thin)	9.36 ± 4.85	26.93 ± 13.41	8.27 ± 1.77	620.28 ± 262.55
Control transversal (thick)	18.10 ± 5.29	15.58 ± 5.93	8.94 ± 2.69	324.34 ± 47.99
Control (acetic acid)	46.11 ± 5.80	24.75 ± 4.13	14.72 ± 0.94	319.51 ± 28.24
Control ethanol	61.65 ± 7.39	27.18 ± 4.76	12.63 ± 1.10	276.26 ± 62.66
Glass fibre mat	23.574 ± 13.21	25.25 ± 12.06	7.36 ± 1.97	799.17 ± 507.04
Thick cocoon-epoxy	121.13 ± 35.02	33.59 ± 11.09	15.84 ± 4.40	158.47
Thin cocoon-epoxy	69.54 ± 54.73	16.1 ± 3.26	28.77 ± 3.80	61.07
Thick cocoon-polyester	43.32 ± 7.02	36.54 ± 5.76	9.78 ± 3.87	649.09 ± 327.56
Thick cocoon-polyurethane	66.414 ± 18.03	26.89 ± 4.18	15.96 ± 3.89	256.82 ± 185.84
Thick cocoon-epoxy-acetic	59.596 ± 14.93	24.35 ± 2.73	16.03 ± 1.83	310.97 ± 76.86
Thick cocoon-epoxy-ethanol	48.93 ± 25.04	19.21 ± 9.32	11.37 ± 5.62	262.97 ± 200.17
Glass fibre mat epoxy	319.96 ± 87.59	72.85 ± 15.14	15.74 ± 6.80	673.7 ± 376.07

APPENDIX 1b

Table	Mechanical test results	for integration of spider and insect silks into single fibre composites

Sample	Max load (mN)	Stress (MPa)	Strain (%)	Young's Modulus (GPa)
Spider control	10 ± 3	1023.68 ± 186.24	23.09 ± 3.99	11.14 ± 1.62
Spider epoxy	20 ± 3.58	97.76 ± 20.42	1.28 ± 0.22	1.28 ± 0.22
Spider polyester	20 ± 3.6	185.25 ± 38.38	2.17 ± 0.58	2.17 ± 0.58
Undeg control	50 ± 6.73	236.97 ± 8.86	1.38 ± 2.93	8.86 ± 1.38
Undeg epoxy	50±18.91	61.69 ± 1.82	1.67 ± 3.86	1.82 ± 1.67
Undeg polyester	70 ± 9.65	88.92 ± 2.61	0.48 ± 4.00	2.61 ± 0.47
Water deg control	80 ± 13.06	164.57 ± 5.53	0.88 ± 4.00	5.53 ± 0.88
Water deg epoxy	90 ± 9.1	117.41 ± 3.40	0.25 ± 3.25	3.40 ± 0.25
Water deg polyester	90 ± 8.71	110.29 ± 3.13	0.58 ± 1.68	3.13 ± 0.58
Soap deg control	30 ± 8.46	274.81 ± 8.79	2.00 ± 4.12	8.79 ± 2.00
Soap deg epoxy	40 ± 4.89	100.82 ± 3.91	0.37 ± 1.84	3.91 ± 0.37
Soap deg polyester	40 ± 14.19	43.93 ± 1.28	0.19 ± 2.62	1.28 ± 0.19
Kevlar control	420 ± 33.5	3374.91 ± 107.40	6.01 ± 0.28	107.40 ± 6.01
Kevlar epoxy	500 ± 160.01	1382.68 ± 46.45	13.95 ± 0.31	46.45 ± 13.95
Carbon control	170 ± 47.1	2257.65 ± 147.07	43.90 ± 0.31	147.07 ± 43.90
Carbon epoxy	160 ± 77.7	812.31 ± 395.30	1.39 ± 0.25	56.79 ± 21.01
Glass control	570 ± 366.82	2718.77 ± 1747.15	2.88 ± 0.48	98.43 ± 55.34
Glass epoxy	400 ± 111.45	475.59 ± 132.39	2.50 ± 0.49	18.57 ± 2.88

Spider Silk as Archetypal Protein Elastomer.

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Abstract

We present an overview of the physical properties of spider silks and introduce a
model designed to study the energy adsorbed by the material as it stretches before
breaking. Of particular interest are the inter- and intra-molecular hydrogen bonds as
well as the role of water in modifying the material properties of silk. A solid
understanding of this interaction is of paramount importance for any deeper insights
into the mechanical properties of any biomaterial. Here we note that the typical
biological material has evolved to function in the fully hydrated i.e. elastomeric state.
We conclude that silk after its transformation from the hydrated feedstock to the de-
hydrated fibre state can in fact be analysed in great detail and interpreted as
representative of a wide range of elastomeric proteins covering, inter alia, bone,
keratins, elastin and collagen.

Introduction

What is silk? What is in the name? The English word 'silk' according to the Oxford
Dictionary derives from a Baltic/Slavic precursor and as such would suggest an old
trading name for a specific type of textile. Today, 'silk' defines primarily the fibre
spun by the larva of the silk-moth *Bombyx mori* and collected by unravelling the
insect's pupal cocoon. But the name 'silk' encompasses other fibres with specific
properties such as filaments produced by caddis-flies, mites and spiders. Even
filaments of the ears of corn are called 'silk'; perhaps because of their 'feel' rather
than any implied biophysical or biochemical similarity with the animal silks?

These classic animal silks, such as the filaments spun by lepidoptera, hymenoptera,
diptera, neuroptera, acarids and araneids, share many traits in their biophysical and
biochemical make-up [1] although there are a fair number of interesting differences [2].
Nevertheless, the spider's formidable dragline silk thread (probably the most
outstanding of biological fibres) is not all that different, at least in its general structure
and in its mechanical properties, from the commercial *Bombyx* silk produced in vast
quantities worldwide. The 'typical' dragline spider silk is considerably stronger and
significantly more extensible than any silkworm silk but both show high initial

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strength that, at a clear yield point, gives way to work hardening [3,4]. Indeed, the difference in strength between silkworm and spider silk can be attributed more to periodic weak points generated during the figure-8 spinning motion of the silkworm, rather than intrinsic structural differences; and these weak spots can be avoided simply by pulling the silk thread away from the worm with a constant force during spinning [5].

Interestingly, people often seem surprised when told that the spider's fibre is a 'silk' just like the fibre of an insect such as the commercial silkworm is a 'silk'. Clearly the public considers the silks of spiders and insects as different materials despite them sharing a name. And public perception has a point. Neither material is what is seems on the surface. For example, the spider makes many different 'silks' extruding from a veritable battery of nozzles around its anus [6] while the insect lays just one thread through its mouth [7,8].

Silkworm silk is a composite fibre (bave) and comprises a doublet of fibroin filaments (brins) surrounded by several thick layers of sericin coating [9,10]. Both fibroin and sericin are proteins, but of very different composition and properties [10]. The two brins, produced and coated in separate ducts, are pressed together while still inside the animal; the sericin hardens in air and typically on the cocoon to form the con-joining bave and tough cocoon wall composite. Spider silk also consists of two filaments, but they are extruded individually and remain easily separated. Their coating is much thinner and consists of a varied number of small molecules of non-protein compounds [11]. This is the composition of 'typical' silk, i.e. the benchmark of spider silks, the dragline and frame silk of the orb weaving tribe of Araneid spiders, such as the garden orb spider Araneus diadematus or the golden silk spider Nephila clavipes.

But both these species, as all other spiders, have more than one type of silk, with the dragline produced by the Major Ampullate (MAA) gland being only one of several different silks, each with specific properties that appear to be optimised to perform key functional roles. Orbweavers like Nephila also produce Minor Ampullate (MIA) silk to accompany the MAA silk in the web, as well as flagelliform silk that forms the core filaments of the orb web's capture thread. The capture thread filaments are coated by yet another 'silk' from the aggregate gland, which is not a hard filament but an aqueous solution of small and highly hygroscopic peptides as well as sticky glycoproteins. The web threads are anchored to the vegetation and affixed to one another by a silk cement originating in the pyriform glands. The eggs are encased in very fine silk filaments from the tubuliform or cylindriform and one type of aciniform gland, while another type of aciniform filaments are used for a multitude of other purposes such as strengthening the cement matrix.

Figure 1 here

Despite this amazing multiplicity of silk types and silk uses, all spider silks, diverse as they are in both mechanical properties and chemical composition, can each and everyone without doubt be called a 'spider silk' because they all derive from one common type of ancestral silk gland. All these glands have a common origin, although over nearly 400 million years of evolution [12] the selection for different functionalities has lead to diversification [13,14] and hence to biochemical tuning [15-18] as well as mechanical specialisations [3,19,20] and temporary tensile adjustments [21,22].

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Thus, the diversity of spider silks shows us the huge variability that is possible for one natural elastomeric biomaterial on both the chemical (genetic) and the physical (mechanical) level. Spider silks therefore open a window into the structure-function relationships of many (perhaps even all) biological protein elastomers.

In the following we will explore the mechanical properties of silks and sketch our reasons for defining 'silk' as a model material to study protein elastomers. As we outline below, our hypothesis is based on a combination of biological, physical and chemical data represented by a wide range of measurements and observations of silk properties and processing over many decades [23-28]. These data illuminate the relationship between composition, structure and properties and thus allow the evolution of a formal silk property model, based on the analysis of the energy management at the molecular level. After all, a biological elastomer has its properties because it has evolved in order to negotiate energy. In the spider's case this is kinetic energy originating from a prey hitting and struggling in the web.

Bio-elastomers typically function either inside the body or at the interface between body and environment. Spider silk is unusual in working away from the animal. Moreover, the typical silk has evolved to function in the dry or only partially hydrated state. For a biological elastomer this is highly unusual (if not unique) because in the body all materials are always bathed in water, and have evolved both functionality and molecular architecture for that fully hydrated stage [29]. Spider silk, which in its natural state encounters a wide range of hydration, therefore provides an interesting (and unique) opportunity to study the importance of water on all levels of bio-elastomer function.

Structure-Function Relationships in Silk

Most of our own work on silk has been the experimental measurements of physical properties from the spinning of the fibre to the final mechanical performance, which has been reviewed previously [30,31]. However, recently this has led us to integrate the findings from other research groups working on the details of silk protein structure in order to develop a self-consistent set of models that relate silk constituents and properties to the apparently complex composition and microstructure of this intriguing material. These new models under development aim to identify the underlying processes that control chemico-physical properties as the design tools for most, perhaps all, natural biomaterials.

Structure-function modelling allowed us to not only map but also to predict the generic space of mechanical properties that are possible in dry silk fibres in the form of stress-strain curves to failure, as illustrated in Figure 1. To this end we deployed the evolutionary concept that in biology 'energy' is the primary 'currency' used by natural selection. Efficient energy management allows animals (and plants) to accumulate reserves, which can ultimately be converted into offspring (the secondary currency). While energy management starts with the basic chemical processes in the cells, it is also a crucial factor in the organism's functional morphology and behaviour. For the spider, web efficiency depends on optimal web engineering in the combination of architecture and silk materials, as well as the choice of a good building site that provides the flies for the spider to catch. These flies arrive with kinetic energy that needs to be distributed in the web not only effectively but also

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efficiently [32], since the silk is a protein that could be used for other critical functions such enhanced offspring production [26].

Silkworm silk is under rather different selection pressures compared to spider silk, as silkworm silk has evolved to make a tough composite and not a tough fibre, which requires a rather different structure-function relationship [5,33-36]. Indeed, the functional requirements of the material *in vivo* must always be considered when studying any biological material, which is typically done *in vitro*. Spider silk provides the ideal biological material for detailed analysis as it must function *ex-vivo* and thus is eminently accessible for detailed and highly relevant studies. Such experimental studies would either be very difficult or impossible for most other biomaterials, which are designed (by nature) to function in the complex context of a highly specific physiological environment. Feather keratin is also a biomaterial designed to function *ex-vivo* but it is grown, rather than spun, which imposes other constraints on its study (as well as its commercial uses), as we shall discuss briefly later.

Returning to the spider's silks, in our model we link the biological rule of 'energy management for survival' to the macromolecular chemistry of silk proteins via the physics of storage and dissipation of mechanical energy at a molecular level. The model has been presented in detail elsewhere [37] and is here only outlined in order to illustrate the elegance of design in natural proteins that allows major changes in property to be controlled by apparently small changes in structure. More recent work has allowed us to develop these models to understand how water changes the properties of silk and to give insights into the mechanical properties of other biological polymers that function in wet environments. These more recent models are introduced for the first time below to suggest how we might predict the full range of properties that are possible in most natural polymers.

The first problem encountered when attempting to model any protein is the need for a quantitative parameter describing of its structure. The peptide sequences of many silks are known and a number of different secondary structure types are possible. However, no clear consensus has yet been established on the detailed crystallographic structures in the different silk types [32 a,b,c]. Rather than define structure in terms of specific space groups, we chose to use 'order' and 'disorder' fractions of peptide segments to quantify the morphology of any specific silk [37]. 'Ordered' segments are aligned sufficiently with neighbours to have two hydrogen bonds between amide groups. Similarly, 'disordered' segments have only one hydrogen bond per amide-amide interaction due to misalignment of adjacent chain segments. This radical simplification allows the role of complex secondary structures in determining mechanical properties to be assigned unambiguously.

To make quantitative predictions of mechanical properties, we chose an approach based upon group interaction modelling, since it had been validated for a wide range of synthetic polymers [38]. With this approach, we can reduce the mechanical functions of the fibre studied to a balance between stiffness and strength that are determined by elastic energy storage, and toughness and extensibility that are determined by energy dissipation. Figure 2 shows the prediction of mechanical properties in graphical form for a typical major silk of *Nephila clavipes* with a model ordered fraction of 66% to illustrate the method.

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Elastic energy is stored as volumetric changes against the bulk modulus, B. Energy is dissipated while the polymer converts mechanical energy irreversibly to heat, mainly because the material goes through transition points such as the glass transitions in the disordered fraction, f_{dis}. These transitions can be quantified by the parameter of loss tangent, $\tan\delta$, which is the ratio of energy lost to energy stored in a deformation cycle. As the polymer is heated or stretched uniaxially, more elastic energy is dissipated and the value of the tensile modulus, E, is reduced in proportion to the residual elastic energy.

In Step one of our analysis [37], we calculate the bulk modulus, B, and loss tangent as a function of temperature. B is calculated using the cohesive energy density as a function of temperature from an ensemble average potential energy function for interactions between all molecular groups in the polymer. The loss tangent is dominated by the two relaxation peaks at around 200 and 470 K, which are treated as two different glass transition events in the disordered fraction; the upper peak is due to amide-amide interactions and the lower peak is due to interactions between the hydrocarbon side chain groups. The specific hydrogen bonding between the amide groups separates these two interactions into distinct relaxation events. The area under the loss tangent peaks (cumulative loss) is predicted using the model parameters for the two interactions, and the distribution is taken typically to be Gaussian with a standard deviation of about 30 degrees.

Step 2 is to calculate the tensile modulus by the cumulative dissipation through the temperature history in the disordered fraction, $f_{dis} \int \tan\delta \, dT$, on B using a relation derived from the coupling of thermal and mechanical energy during deformation

$$ E \approx B \exp\left(-\frac{f_{dis} \int \tan\delta \, dT}{A \, B} \right) \tag{1} $$

where $A = 1.5 \text{ GPa}^{-1}$ is a term that comprises dimensional parameters for the ensemble average model peptide segment. The relation shows that E is higher for a higher ordered fraction, since B is higher due to the higher cohesive energy with more hydrogen bonding and the cumulative loss is lower due to the reduced transition events in the smaller disordered fraction. The predicted dynamic mechanical properties of silk with an ordered fraction of 66% from steps 1 and 2 are shown in Figure 2a, with experimental data for a major ampullate silk shown for comparison.

Step 3 is to calculate strain, ε, and stress, σ, in a self-consistent pair of equations using a dummy variable of temperature (that has a value T_ε at a combination of ε and σ) and the linear thermal expansion coefficient, which is predicted to be $\beta \approx 0.0001 \text{ K}^{-1}$.

$$ \varepsilon = \int_T^{T_\varepsilon} \beta \, dT \quad , \qquad \sigma = \int_T^{T_\varepsilon} \beta \, E \, dT \tag{2} $$

Figure 2b shows the predicted stress-strain profiles for a number of values of ordered fraction that are typical within the range seen in spider silk fibres. Key features are the yield strain, $\varepsilon_y \approx 0.02$, determined by the amide-amide relaxation peak temperature, and the post-yield strain hardening to failure, which is due to the

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transformation of the post-yield rubberlike states to crystal and glassy states until all the ductile material has been converted to a brittle form, at which point the fibre breaks. Figure 2c shows experimental stress-strain plots for *Nephila* major fibres spun under different conditions in order to generate a broad range of mechanical properties for comparison with the model predictions [39].

Thus, we can predict the full range of possible stress-strain profiles in dry silk simply by changing the fraction of ordered segments. Typical values of ordered fraction for different silk types are 0.85 for *Bombyx mori* cocoon silk, 0.66 for major dragline silk, and 0.2 for flagelliform. Generally, higher initial modulus (stiffness) gives a higher failure stress (strength) but lower strain to failure (elongation) and area under the stress-strain curve (toughness).

Figure 2 here.

Water Sensitivity and Cyclical Loading

An important characteristic of different silks is their sensitivity to water. Some silks shrink considerably upon exposure to water, which is called supercontraction [40,42-49] At the same time as shrinking, these silks also show a large drop in their elastic modulus and a commensurate increase in their strain to failure after they have been dried out again [50,51]. Major silk is sensitive to water, whereas and minor silk from the same spider is not [45]. Understanding this selective response to water is not only important scientifically in order to understand silk properties overall but may also be very useful commercially if one wants to deploy such silks as implantable biomaterials.

The experimental stress-strain curves in Figure 2c are major silks with different degrees of supercontraction strain, C_{sh}, that are labelled on the curves. Inspection of the disordered fraction in the model curves of Figure 2b and supercontraction strain in the experimental curves of Figure 2c suggests a direct numerical relation between these parameters.

$$C_{sh} \equiv f_{ord} - 0.45 \qquad (3)$$

Although the precise numerical identity between C_{sh} and the change in Δf_{dis} is coincidence, the identity can be explained quantitatively by comparing the mechanical energy of the strain C_{sh} at the yield stress with the energy to convert the fraction Δf_{dis} of disordered states to ordered states above yield during strain hardening of a fibre. Thus, supercontraction is directly linked to the change (loss) of order in a silk on exposure to water and the fully supercontracted state has an ordered fraction of about 0.45. The maximum order inferred from the stiffest silk stress-strain curves suggests a minimum $f_{dis} \approx 0.15$

When one first dries and then elongates supercontracted silk, a fraction of the elongation is retained in the fibre and can again be recovered by another exposure to water [40,50,52]. The inset in Figure 3 shows repeated cycles of strain applied to supercontracted major silk, with modulus increasing as the fibre length increases. This cyclical loading characteristic shows that some degree of order in major silk is reversible, and suggests that the ordered phase of a specific fraction of the peptide

segments can be manipulated by exposure to water. In nature, this phenomenon may be useful, as dew condensation can lead to the tightening of webs that have sagged. In effect, this behaviour allows a specific silk (and by extension the web where it is deployed) to perform optimally under the full range of vapour pressures (ambient humidities) encountered from the desert to the rain forest and from the late night to the middle of the day [53,54]

Figure 3 shows model plots of cyclical stress-strain loading of a fully supercontracted major silk, where each new loading starts with the origin in the strain axis shifted by the supercontraction strain predicted by the change in f_{dis} during strain hardening in the previous cycle. Agreement with the experimental plots in the inset is good, and validates the general principle. We are currently modelling the rate of relaxation of strain at the end of each loading cycle by making the relaxation rate a function of the fraction of rubber-like states at each point in the relaxation process. This will allow the full loading-unloading profile to be predicted and also refine the model for stress-strain response around the yield point, where some dynamic stress relaxation is seen in the lower modulus fibres.

Figure 3 here

Model Structure of Major Silk

Using the model for mechanical properties and water sensitivity, we are in a position to formulate a model structure for dragline MAA silk that is based on both the measured chemical composition (based on analytical studies) and the degree of order (deduced from the model stress-strain response). The response to water of the MAA (and other silks) makes it apparent that the rather simple parameters of order and disorder need to be refined more. Not least because a recent study on supercontraction and its effects on mechanical properties [40,41] has demonstrated that some degree of order can be manipulated by a combination of spinning conditions and hydration.

Major ampullate MAA silk is generally taken to be an alloy of Spidroins I and II, which we take here to have characteristic segment compositions [55-58],

Spidroin I: GQG GYG GLG SQG A GRG GLG GQG A GAAAAAAAGG A

Spidroin II: GPGGY GPGQQ GPGGY GPGQQ GPGGY GPGQQ GPSGPGS AAAAAAAAAA

Taking a number average proline fraction of about 6% suggests a ratio of about 2:1 for Spidroin I to Spidroin II segments, with a weight fraction of about 33% for Spidroin II.

The important feature of proline is that it is not intrinsically 'ordered', since the ring in the chain backbone twists the torsional angles away from any of the simple configurations that can be ordered and it has no amide group for two potential hydrogen bonds. Each proline group is associated with four other peptide groups in the pentamer sequence GPGXX, so these four groups are intrinsically 'disordered' by the proline. Nevertheless, they are potentially capable of 'order' under the correct set of packing conditions and an approximate calculation of the GGXX groups in the

GPGXX pentamers suggests about 20% by volume/weight of the total major silk composition is in this state. Since each Spidroin II chain interacts in a pairwise manner with Spidroin I segments in the polymer alloy, this suggests about 40% of the total amide-amide interactions in major silk are intrinsically disordered (defined by inter-amide hydrogen bonds), but with the potential for order.

Studies of molecular structure in silk have suggested that silk has a folded hairpin structure [59] [60] with about 6 peptide segments per fold, shown in a simplified form in Figure 4. If we take the peptide segment at a turn of the hairpins as being unable to form ordered interchain hydrogen bonds, this suggests a fraction of about 0.17 of the segments as being permanently disordered. Using this simple argument, three types of material in the major silk can be suggested with approximate fractions in the hairpin-turn model for silk nanofibrils as a 'string of beads':

- permanent disorder: hairpin turns: about 0.15 fraction
- intrinsically disordered but with a potential for order under strain: about 0.4 fraction located between turns due to Spidroin II interactions
- permanent order: Spidroin I between turns: about 0.45 fraction.

Figure 4 here

Thus, major MAA dragline silk has an underpinning set of mechanical properties that are determined by the permanent order and disorder fractions and are those of supercontracted fibres. Further order can be forced into the structure by greater stress during the spinning process and gives the spider considerable flexibility in controlling mechanical properties. Minor MAI dragline silk does not contain proline [65] and is not water sensitive [66], but we do not yet have enough structural information to allow us to model its properties in any detail.

Before moving on to more general aspects of other biomaterials, it is interesting to consider the properties of pure Spidroin II fibres with its high proline content. These are predicted here to have properties characteristic of flagelliform silk in the hydrated supercontracted state and potentially be almost as stiff as silkworm fibres in the fully ordered dry state, but with a high sensitivity to water and deformation conditions. Such fibres [Nexia 'biosteel' silk] were spun from recombinant silk 'cloned' from Spidroin II and indeed show [67] the properties expected from our model.

Silk Compared to Other Biopolymers

Since silks are produced by spinning, rather than by growth, they have a realistic potential for commercial bulk production. Of specific interest (because of the inherent biocompatibility of spidroin filaments) are fibres for medical applications such as tendon or ligament replacements, where a synthetic silk material should be gradually replaced in the body by natural collagenous protein fibres. The mechanical properties of the silk can be matched to those of tendons by using classical rope making skills [68], and the biodegradation rate will be determined largely by the hydration of the silk. Since mechanical properties and hydration both depend upon the

fraction of disordered peptide segments, balancing all these aspects presents an interesting challenge. Silk has also been suggested as a template for bone growth, where hydroxyapatite grows around synthetic silk fibres, and the natural tropocollagen-hydroxyapatite structure develops with time [68].

Spiders do produce elastomeric (viscid) silk with a modulus of the order 1 MPa, which has the mechanical properties of a rubberlike polymer. This web capture-silk is a composite of a Flagelliform (FLG) core fibre and an Aggregate coating, with the latter providing hygroscopic components that keep the silk fully hydrated even under rather dry ambient conditions [43,53]. The water of the coat penetrates and plasticizes the filament protein and reduces its glass transition temperature to below ambient and thus induces a rubber-like state in the polymer [69]. Such deeply hydrated silk material (not only FLG but also MAA) has mechanical properties that compare with mammalian elastin. Elastin is an important biopolymer, with a range of biological functions, one of which (most importantly) keeps our blood-pressure in healthy range through its action in our arteries [29].

Collagen is an equally important, and much more widely spread, bio-polymer making up (in its hydrated form) over 80% of our body's weight and much of its functional morphology. Accordingly, silks with collagen-behaviour or conducive to collagen infiltration are of great interest to bio-medical silk-researchers. The structure and properties of such important bio-functional polypeptides as collagen, elastin and a range of others such as feather keratin and mussle byssus thread have been studied extensively for quite some time [70,71] with many exciting developments in recent years [29,72,73]. Nevertheless, at present their chemical composition and physical morphology still elude the full quantitative explanation and accurate prediction of mechanical properties. It would be very useful indeed if our studies on silks and the models of silk structure-function relations outlined above could guide us also to a better understanding of these other natural biomaterials. The materials have evolved to work fully hydrated and deep inside the body or as part of the body's interface with the environment. And because of this interaction with the body physiology they are not easy to study quantitatively. Silk, in contrast, has evolved to work away from the body and thus can provide us with the experimental data required to establish it as the perfect general model for these other, more intractable, fibres and fibre composites.

Indeed, a full understanding of natural silks should lead to the design of 'silk' analogues of a wide range of other protein biopolymers with elastic properties ranging from the 20 GPa of bone to the MPa modulus of protein elastomers such as elastin and the kPa modulus of resilin at low strain. Of equal importance to forming the link from silk to other bio-fibres, perhaps, is to understand the highly nonlinear strain dependence of mechanical properties that are crucial for biological function; for example, the stiffening of collagen from 1 MPa to 1 GPa modulus after a few percent strain, or that of elastin at about 100% strain.

To quantify the effects of water is a key step for modelling most structural proteins, as water is not only their natural environment, but it is also Biology's principal plasticizing agent [74,75]. We discussed above that water affects only disordered segments; and it is reasonable to assume that one water molecule can hydrogen bond to each disordered amide group, which is a weight fraction of 0.29 water in the water-amide pair. Using either the group interaction modelling relation or the Fox-Flory

rule [38] for calculating the glass transition temperature of the water-amide pairs (using T_g values of 160 and 473K for water and disordered amide respectively) suggests T_g = 300K for the hydrated segment [37]. As more water hydrates a disordered protein at low water levels, then a greater fraction must operate above its upper glass transition temperature at normal body temperature and behave as a rubber. Of course, excess water is another matter, and we will consider this later.

Let us look at keratin first as it is also a biomaterial evolved to function outside the body and in the dry state. Keratin is a semi-crystalline composite material with about 0.3 - 0.4 fraction of ordered crystal fibrils dispersed in a disordered matrix [76]. Using the silk model relation to predict the isotropic tensile modulus of dry keratin with this range of ordered segments gives an expected range of 5.5 to 6.5 GPa, and the yield strain of unhydrated segments is unchanged at 2%, which agrees with observation. As more water hydrates the disordered matrix fraction at increasing levels of relative humidity, a slightly more complex calculation to include the hydrated segments with a new contribution to loss at T_g = 300K shows that the modulus reduces with increasing hydrated fraction, until a lower limit of between 0.5 and 1 GPa is reached when all the disordered segments have been hydrated at 100% RH. This is in line with the 0.5 GPa modulus measured for wet hoof keratin [77]. We note that the hydrated modulus value is calculated at the glass transition temperature of 300 K, and infers that the material should have a very high damping capability while operating in the glass transition zone.

Collagen, unlike keratin, has evolved to work inside the body and in the wet state. But it is also a semi-crystaline composite material with comparable (to keratin) fractions of order and disorder. Bulk collagen consists of a left handed triple helix with a pitch of about 10 nm that contains about 35% glycine (every third segment to stabilise the helix) and 21% proline or hydroxyproline. Since the characteristic periodicity of the peptide chains is dominated by the glycine into trimer groups, a reasonable estimate for the degree of order in the structure is again about 0.35 from the likely hydrogen bonding with the small glycine groups in the helix and the disordering influence of proline. Like keratin, the predicted dry modulus is about 6 GPa, and the hydrated modulus is again about 1 GPa, as measured [77a]. Note that here we have not included the low initial modulus of the collagen due to proteoglycan coating of the collagen fibrils [78,79]. Recently a discussion of collagen in bone has been published outlining how the polymer-mineral nanostructure of bone imparts its attractive combination of stiffness and toughness [37].

Elastin, has an even more complex structure than collagen albeit being largely disordered [29,80-82]. Its principal component– the (PGVGV) segment – takes a square wave form of β-turns forming a primary helix with a diameter of about 2.4 nm [83]. This primary helix is then twisted again into a triple helix with a pitch of about 7.2 nm. We note the similarity of the elastin pentamer with Spidroin II. Hydrated elastin has excess water (about 35% by weight) above that required to hydrogen bond simply with the amide segments. This affects the glass transition temperature by reducing it well below operating conditions, such that elastin has a rubberlike modulus of about 1 MPa. Taking hydrated elastin to be fully disordered, the calculated bulk modulus of 3.5 GPa (with 35% water) and the usual cumulative loss of 45 through the glass transitions predict a tensile modulus of 1 MPa using the elastic modulus relation of equation 1.

The range of mechanical properties provided by silk, keratin, collagen and elastin is extended upwards in modulus by mineralization in materials such as bone, which is a natural hybrid nanocomposite of hydroxyapatite mineral in a hydrated tropocollagen polymer matrix [84]. These materials range from mineralised tendon, through various mineral fractions of bone, to tooth enamel from about 10 to 30 GPa modulus respectively [85]. Porter [37] has published a model for structure-property relations in bone that is consistent with the model for silk reviewed above, so the same underlying mechanisms operating in silk can be used as a general template for understanding a wide range of biomaterials.

Summary and Challenges

The overview presented here shows not only why silk functions it does, but also how silk can be used to provide insights into other important structural proteins at both a practical and theoretical level. This leads us to conclude that silk can be considered as an ideal archetypal elastomeric protein. The range of mechanical properties that can be derived from silk-based proteins is enormous; from mineralised bone-like materials with a modulus of 20 GPa, through classical silks with a modulus 2 – 10 GPa, down to viscid elastin-like rubbers with only 1 MPa.

The outlined combination of experimental work with analytical modelling shows the large range of mechanical properties that silk can assume with apparently small changes in chemical structure and processing. This makes silk an important analytical tool as well as an interesting bio-polymer for a wide range of important applications. In this context silk can provide a valuable basis for complex protein composites. For example, subtle combinations of protein structures with other biopolymers can produce elegant materials such as peptidoglycans for the walls of bacterial cells, which are rigid impermeable polymers when dry, but swell by hydration of the protein segments between the disaccharides to form a tough semipermeable membrane; for example, murein as the walls of virus cells [86,87].

Now that we have begun to understand the interaction of spinning and material properties in silk, as well as its underlying principles, it is the next great challenge to produce silk fibres and films in a biomimetic and environmentally benign manner from aqueous dope suspensions. Although silk fibres can without much difficulty be pulled straight from both spider and silkworm dope (our own observations), and fibres with good material properties can be spun relatively easily from regenerated silk[68] yet making semi-natural fibres from natural, genetically modified or indeed synthetic feedstock with properties comparable to the natural fibre is far from solved [57,88-90]. After all, the natural spinning process is a complex combination of material chemistry and rheological processing [24,91,92]. Nevertheless, nature shows us how it could be done and by working on all aspects of understanding the biological spinning process in different species through to theoretical models for the complex visco-elastic properties of the dope we should have a good chance of success to unravel the secret of the spider's silk.

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Figure legends

Figure 1. *Nephila* golden silk spider showing its silk glands as well as the use for each silk and its mechanical properties in the form of comparative experimental stress-strain plots, where the highlighted line in each graph is that of the graph label; the symbols D and W in the Flagelliform graph are dry and wet respectively. We thank Chris Holland for the artwork.

Figure 2. Model for the mechanical properties of silk: 2a shows a comparison of experimental dynamic mechanical analysis of elastic modulus and loss tangent (points) with model predictions (lines) [37]; 2b shows model predictions of stress-strain relations for a range of realistic ordered fractions in silk; 2c shows experimental stress-strain plots for a spider silk spun under different conditions [39-41].

Figure 3. Cyclical loading plots; the main plots model predictions of experimental data [50] shown in the inset (see text for details).

Figure 4. A fibroin folded chain backbone and a simplified hairpin fold morphology of spider silk.

References

1. Craig, C. *Ann. Rev. Entomol.* **42**, 231-267 (1997).
2. Craig, C. L. *Spiderwebs and silks: tracing evolution from molecules to genes to phenotypes* (Oxford University Press, New York, 2003).
3. Denny, M. W. in *The Mechanical Properties of Biological Materials* (eds. Vincent, J. F. V. & Currey, J. D.) 245-271 (Soc. Exp. Biol. Symp. 34., Cambridge University Press, Cambridge UK, 1980).
4. Vollrath, F. & Porter, D. *Appl. Physics A* **82**, 205-212 (2006).
5. Shao, Z. Z. & Vollrath, F. *Nature* **418**, 741-741 (2002).
6. Vollrath, F. Spider webs and silks. *Scientific American*, 70-76 (1992).
7. Akai, H. *Experientia* **39,** 443-449 (1983)
7 a. Akai, H. pp 219-253 in Microscopic Anatomy of Invertebrates. Wiley-Liss, New York, (1988)
8. Magoshi, J., Magoshi, Y. & Nakamura, S. pp 292-310 in *Silk Polymers. Materials Science and Biotechnology* (eds. Kaplan, D., Adams, W. W., Farmer, B. & Viney, C.) (American Chemical Society, Washington, 1994).
9. Asakura, T. & Kaplan, D. L. Silk production and processing. *Encycl. Agricult. Sci.* **4**, 1-11 (1994).
10. Perez-Rigueiro, J., Viney, C., Llorca, J. & Elices, M. *J. Appl. Polymer Sci.* **75**, 1270-1277 (2000).
11. Vollrath, F. et al. *Nature* **345**, 526-528 (1990).
12. Shear, W. A., Palmer, J. M., Coddington, J. A. & Bonamo, P. M. A *Science* **246**, 479-481 (1989).
13. Shultz, J. W. *Biol. Rev.* **62**, 89-113 (1987).
14. Gosline, J., Guerette, P. & Ortlepp, C. *Proc SPIE Int Soc Opt Eng* **2716**, 296-299 (1996).
15. Hayashi, C. Y. & Lewis, R. V. *Science* **287**, 1477-1479 (2000).
16. Garb, J. E. & Hayashi, C. Y. *PNAS* **102**, 11379-11384 (2005).
16a. Gatesy, J., C. Hayashi, Motrluk D., Woods, J. & Lewis, R. *Science* **291**: 2603-2605 (2001).
16b. Gosline, J. M., Guerette, P. A., Ortlepp, C. S., & Savage, K. N. *J. Exp. Biol.* **202,** 3295-3303 (1999).
17. Tian, M. & Lewis, R. V. *Biochem.* **44**, 8006-8012 (2005).
18. Brooks, A. E., Steinkraus, H. B., Nelson, S. R. & Lewis, R. V. *Biomacromol.* **6**, 3095-3099 (2005).
19. Vollrath, F. & Köhler, T. *J. Exper. Zool.* **271**, 1-17 (1995).
20. Vollrath, F., Madsen, B. & Shao, Z. Z. *Phil. Trans. Roy. Soc. London Series B-Biol. Sci* **268**, 2339-2346 (2001).
21. Madsen, B., Shao, Z. Z. & Vollrath, F. *International Journal of Biological Macromolecules* **24**, 301-306 (1999).
22. Garrido, M. A., Elices, M., Viney, C. & Perez-Rigueiro, J. *Polymer* **43**, 4495-4502 (2002).
23. Gosline, J. et al. in *Elastomeric Proteins* (ed. Bailey, A. J.) 15-38 (Cambridge University Press, Cambridge, 2005).
24. Vollrath, F. & Knight, D. P. *Nature* **410**, 541-548 (2001).
25. Vollrath, F. & Knight, D. in *Biotechnology of Biopolymers; from synthesis to patents Volume 2* (eds. Steinbuchel, A. & Doi, Y.) 873-894 (Wiley-VCH, 2005).

26. Craig, C. L. *Spiderwebs and silks: tracing evolution from molecules to genes to phenotypes* (Oxford University Press, New York, 2003).

27. Winkler, S. & Kaplan, D. L. *Rev. Molec. Biotechnol.* **74**, 85-93 (2000).

28. Sponner, A. et al. *Biochemistry* **44**, 4727-4736 (2005).

29. Gosline, J. et al. in *Elastomeric Proteins* (eds. Shewry, P. R., Tatham, A. S. & Bailey, A. J.) 15-38 (Cambridge University Press, Cambridge, 2003).

30. Vollrath, F. & Knight, D. P. in *Elastomeric Proteins* (eds. Shewry, P. R., Tatham, A. S. & Bailey, A. J.) 152-174 (CUP, Cambridge, 2003).

31. Knight, D. P. & Vollrath, F. in *Elastomeric Proteins* (eds. Shewry, P. R., Tatham, A. S. & Bailey, A. J.) 115-135 (CUP, Cambridge, 2003).

32. Lin, L. H., Edmonds, D. T. & Vollrath, F. *Nature* **373**, 146-148 (1995).

32 a. Riekel, C, Müller M,Vollrath F, *Macromol.* 32: 4464-4466 (1999)

32 b. Riekel C, Madsen B, Knight DP, Vollrath F, *Biol. Macromol.* 24: 179-186 (2000)

32 c. Riekel C, Vollrath F, *Int. J. Biol. Macromol.* 29: 203-210 (2001).

32 d. Riekel C, Roessle M, Sapede D, Vollrath F Naturwissenschaften xx:30-33 (2004).

33. Perez-Rigueiro, J., Viney, C., Llorca, J., and Elices, M. *J. Appl. Polymer Sci.* **70,** 2439-2447(1998).

34. Perez-Rigueiro, J., Viney, C., Llorca, J., and Elices, M. Mechanical properties of single-brin silkworm silk. *J. Appl. Polymer Sci.* **75,** 1270-127 (2000).

35. Perez-Riguero, J., Elices, M., Llorca, J. & Viney, C. *J. Appl. Polymer Sci.* **82,** 2245-2251 (2001).

36. Perez-Rigueiro, J., Elices, M., Llorca, J. & Viney, C. *J. Appl. Polymer Sci.* **84,** 1431-1437 (2002).

37. Porter, D., Vollrath, F. & Shao, J. Z. *Europ. Physical J. E* **16**, 199-206 (2005).

38. Porter, D. *Group Interaction Modelling of Polymers* (Marcel Dekker, New York, 1995).

39. Yang, Y. et al. *Adv. Mat.* **17**, 84-+ (2005).

40. Liu, Y., Shao, Z. & Vollrath, F. *Nature Mat.* (2005).

41. Liu, Y., Shao, Z. Z. & Vollrath, F. *Chemical Comm.* **19**, 2489 - 2491 (2005).

42. Work, R. W. & Morosoff, N. A *Textile Res. J.* **52**, 349-356 (1982).

43. Vollrath, F. & Edmonds, D. T. *Nature* **340**, 305-307 (1989).

44. Vollrath, F., Bonthorne, K. M., Hunter, B. K. & Sanders, J. K. M. *Proc. R. Soc. Lond.* **248**, 141-144 (1992).

45. Vollrath, F., Holtet, T., Thogersen, H. C. & Frische, S. *Proc. Roy. Soc. Lond.* **263**, 147-151 (1996).

46. Grubb, D. T., Jackrel, D. B. & Jelinski, L. W. *Abstracts American Chemical Society* **214**, 164- (1997).

46a Grubb, D. T., Jelinski, L. W. *Macromol.* **30**, 2860-2867 (1997).

46b Grubb, D. T. and J. Gending *Int. J. Biol. Macromol.* **24**: 203-210 (1999).

47. Jelinski. *JACS* **122**, 9019-9025 (2000).

48. Bell, F. I., McEwen, I. J. & Viney, C. *Nature* **416**, 37-37 (2002).

49. Perez-Rigueiro, J., Elices, M. & Guinea, G. V. *Polymer* **44**, 3733-3736 (2003).

50. Shao, Z. Z. & Vollrath, F. *Polymer* **40**, 1799-1806 (1999).

51. Shao, Z., Vollrath, F., Sirichaisit, J. & Young, R. J. *Polymer* **40**, 2493-2500 (1999).

52. Eles, P. T. & Michal, C. A. *Macromol.* **37**, 1342-1345 (2004).

53. Edmonds, D. T. & Vollrath, F. *Proc. Roy Soc. Lond.* **248**, 145-148 (1992).

54. Vollrath, F. in *Plastics in automotive engineering* (ed. Vollrath, L.) 1-16 (VDI Verlag, Düsseldorf, 1993).

55. Lewis, R. V., Hinman, M., Kothakota, S. & Fournier, M. J. *Protein Express. Purif.* **7**, 400-406 (1996).

56. Winkler, S. et al. *Int J Biol Macromol* **24**, 265-270 (1999).

57. Fahnestock, S. R. in *Biotechnology of Biopolymers; from synthesis to patents Volume 2* (eds. Steinbuchel, A. & Doi, Y.) 895-927 (Wiley-VCH, 2005).

58. O'Brien, J. P., Fahnestock, S. R., Termonia, Y. & Gardner, K. C. H. *Adv. Mat.* **10**, 1185-1195 (1998).

59. Simmons, A. H., Michal, C. A. & Jelinski, L. W.*Science* **271**, 84-87 (1996).

60. Hayashi, C. Y., Shipley, N. H. & Lewis, R. V. *Int J Biol Macromol* **24**, 271-275 (1999).

61. Beek, v. J. D., Hess, H., Vollrath, F. & Meier, B. H. *PNAS USA* **99**, 10266-10271 (2002).

62. Valluzzi, R. & Jin, H. Y. *Biomacromol.* **5**, 696-703 (2004).

63. Krejchi, M. T. et al. *Macromol.* **30**, 5012-5024 (1997).

64. Peng, X. et al. *Biomacromol.* **6**, 302-308 (2005).

65. Colgin, M. A. & Lewis, R. V. *Protein Science* **7**, 667-672 (1998).

66. Vollrath, F. & Knight, D. P. *Int J Biol Macromol* **24**, 243-249 (1999).

67. Lazaris, A. et al. *Science* **295**, 472-476 (2002).

68. Altman, G. H. et al. *Biomaterials* **24**, 401-416 (2003).

69. Gosline, J. M., Denny, M. W. & Demont, M. E. *Nature* **309**, 551-552 (1984).

69a Gosline, J. M., Pollack, C. C., Guerette, P. A., Cheng, A. DeMont, M. E., Denny, M. W. in *Silk Polymers. Materials Science and Biotechnology* (eds. Kaplan, D., Adams, W. W., Farmer, B. , Viney, C.) pp 328-341 (American Chemical Society, Washington) (1994).

70. Vincent, J. F. V. *Biomechanics Materials* (OUP, Oxford, 1992).

71. Vincent, J. F. V. in *Elastomeric Proteins* (eds. Shewry, P. R., Tatham, A. S. & Bailey, A. J.) 366-378 (CUP, Cambridge, 2003).

72. Shewry, P. R., Tatham, A. S. & Bailey, A. J. *Elastomeric Proteins* (CUP, Cambridge, 2003).

73. Waite, J. H., Vaccaro, E., Sun, C. & Lucas, J. in *Elastomeric Proteins* (eds. Shewry, P. R., Tatham, A. S. & Bailey, A. J.) (CUP, Cambridge, 2003).

74. Vincent, L. S. (1985).

75. Franks, F. *Biophysical Chemistry* **96**, 117-127 (2002).

76. Fraser, R. D. B. & Macrae, T. P. in *The mechanical Properties of Biological Materials* (eds. Vincent, J. F. V. & Currey, J. D.) 211-246 (CUP, Cambridge, 1980).

77. Bertram, J. E. & Gosline, J. M.. *J Exp Biol* **125**, 29-47 (1986).

77a Vincent, J *Structural Biomaterials.* Princeton University, Princeton (1990).

78. Fratzl, P. et al. *J. Struct. Biol.* **122**, 119-22 (1997).

79. Fratzl, P. in *Elastomeric Proteins* (eds. Shewry, P. R., Tatham, A. S. & Bailey, A. J.) 175-188 (CUP, Cambridge, 2003).

80. Gosline, J. M. *Symp Soc Exp Biol* **34**, 332-57 (1980).

81. Aaron, B. B. & Gosline, J. M. *Biopolymers* **20**, 1247-1260 (1981).

82. Bellingham, C. M. & Keeley, F. W. in *Elastomeric Proteins* (eds. Shewry, P. R., Tatham, A. S. & Bailey, A. J.) 39-53 (CUP, Cambridge, 2003).

83. Urry, D. W. *Ultrastruct Pathol* **4**, 227-51 (1983).

84. Katz, J. L. in *The mechanical Properties of Biological* (eds. Vincent, J. F. V. & Currey, J. D.) 137-168 (CUP, Cambridge, 1980).

85.   Currey, J. D. *Phil. Trans. Roy. Soc. London Series B-Biol. Sci.* **304**, 509-18 (1984).
86.   Rogers, H., Perkins, H. R. & Ward, J. B. *TITLE Chapter 6* (Chapman and Hall, London, 1980).
87.   Thwaites, J. J. & Mendelson, N. H. *Int J Biol Macromol* **11**, 201-6 (1989).
88.   Cappello, J. & McGrath, K. P. in *Silk Polymers. Materials Science and Biotechnology* (eds. Kaplan, D., Adams, W. W., Farmer, B. & Viney, C.) 311-327 (American Chemical Society, Washington, 1994).
89.   Karatzas, C. N. et al. in *Biotechnology of Biopolymers; from synthesis to patents Volume 2* (eds. Steinbuchel, A. & Doi, Y.) 945-965 (Wiley-VCH, 2005).
90.   Scheller, J. & Conrad, U. in *Biotechnology of Biopolymers; from synthesis to patents Volume 2* (eds. Steinbuchel, A. & Doi, Y.) 929-943 (Wiley-VCH, 2005).
91.   Knight, D. P. & Vollrath, F. *Phil. Trans. Roy. Soc. London Series B-Biol. Sci* **357**, 155-163 (2002).
92.   Kerkam, K., Viney, C., Kaplan, D., and Lombardi, S. *Nature 349,* 596-598 (1991).
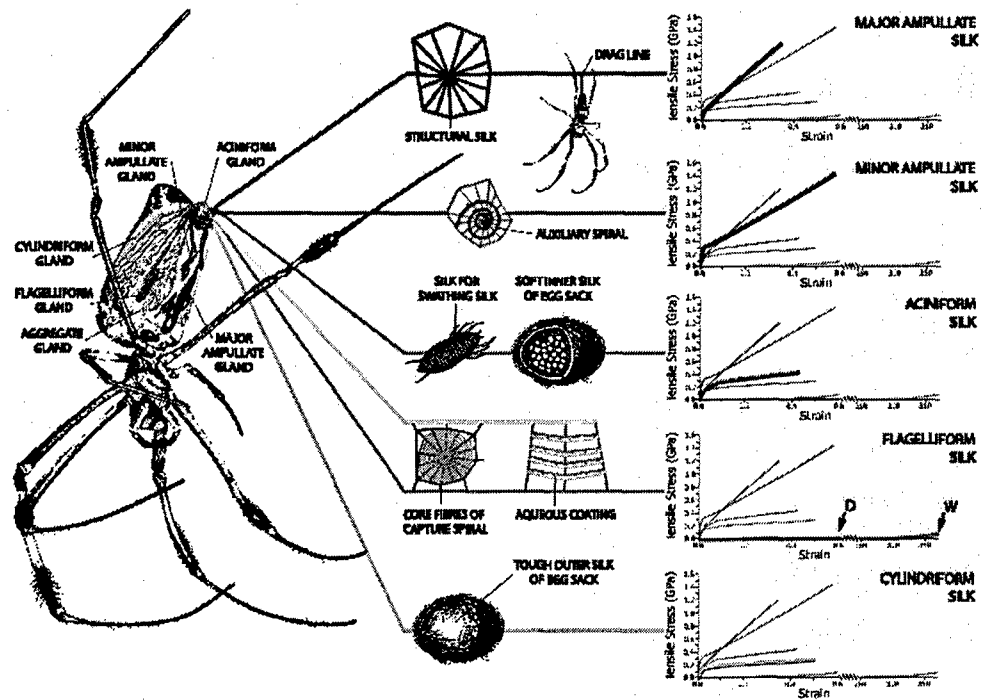
FIGURE 1

Figure 1. *Nephila* golden silk spider showing its silk glands as well as the use for each silk and its mechanical properties in the form of comparative experimental stress-strain plots, where the highlighted line in each graph is that of the graph label; the symbols D and W in the Flagelliform graph are dry and wet respectively. We thank Chris Holland for the artwork.
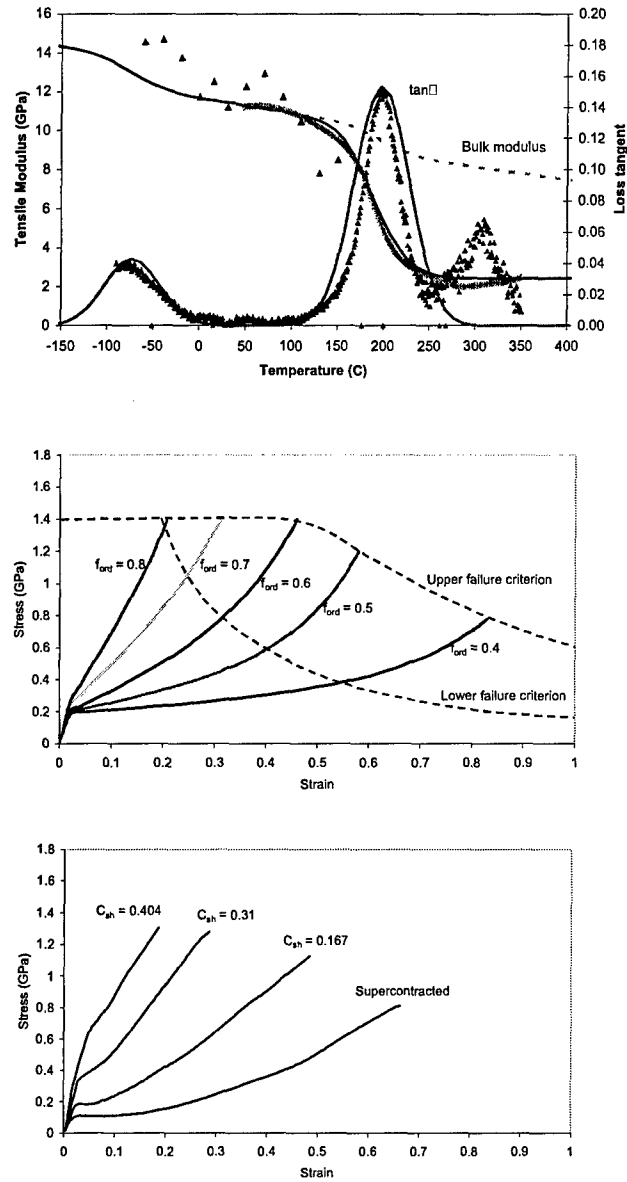
FIGURE 2

Figure 2. Model for the mechanical properties of silk: 2a shows a comparison of experimental dynamic mechanical analysis of elastic modulus and loss tangent (points) with model predictions (lines) [37]; 2b shows model predictions of stress-strain relations for a range of realistic ordered fractions in silk; 2c shows experimental stress-strain plots for a spider silk spun under different conditions [39-41].
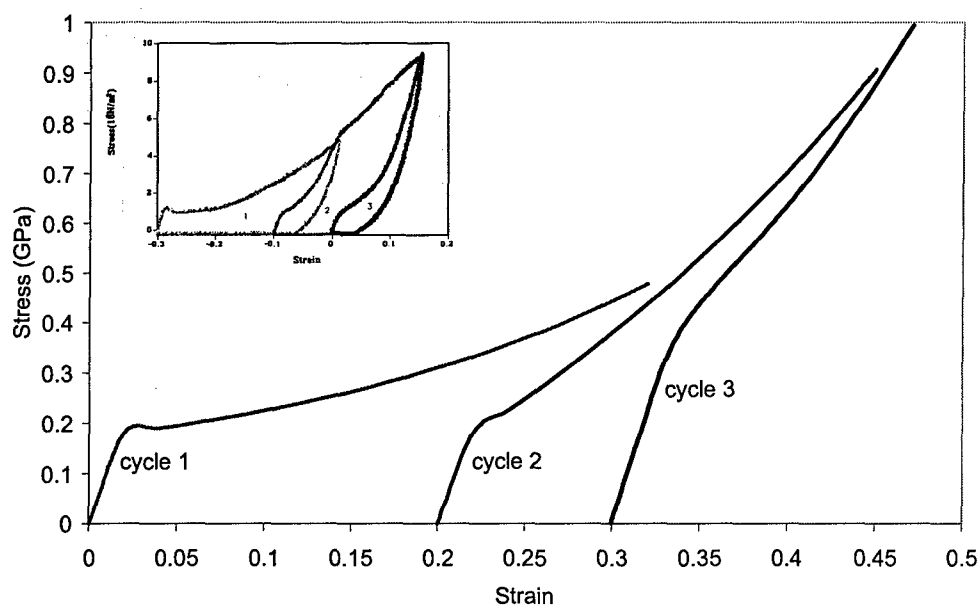
FIGURE 3

Figure 3. Cyclical loading plots; the main plots model predictions of experimental data [50] shown in the inset (see text for details).
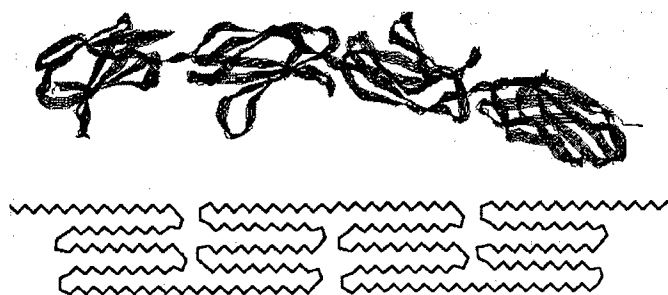
FIGURE 4

Figure 4. A fibroin folded chain backbone and a simplified hairpin fold morphology of spider silk.