



SILK: RECONSTITUTION AND NOVEL 3D COMPOSITE

**Fritz Vollrath
THE UNIVERSITY OF OXFORD**

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Final Report**

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14. ABSTRACT The project examines 2 important aspects of silk : the spinning of quality fibers from a dope and the use of silks in functional composites. Using the spider's spinning techniques we were able to spin high quality spider-type filaments from silkworm dopes energy efficient and with water as only solvent. Using elephant ivory and rhino horn as models we were able to make tough and/or light weight bio-inspired composites based on a pure silk or a silk&cellulose matrix.					
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3rd year report Aug 2017 to Aug 2018 and also full award publication list

Overview: in this project we examine 2 interesting aspects of silk. In the work packages *molecular interactions* we examine silk reconstitution with the view of invoking the aquamelt concept to produce high quality RSF (Reconstituted Silk Fibroin) liquid dope that behaves more like native silk (NSF) than common RSF. In the work packages *mineral/silk composites* we aim to make a tough hydroxyl apatite based silk composite bio-inspired by elephant ivory. Both work packages are closely integrated. WP 'dope' is more focused on silk- transitions but with the ultimate practical aim to provide materials for further processing while WP 'horn/ivory' uses such materials in the development of novel, tough silk composites. In this reporting period I focus on aquamelt spinning spider-like silk filaments from a bespoke liquid dope derived from natural silk material of the mulberry silkworm. focused on understanding native and reconstituted liquid silk dopes and the role of silk filaments in a wide range of composites. I shall also report on our studies on silk-based faux rhino-horn while the work on the faux ivory is still very much ongoing.

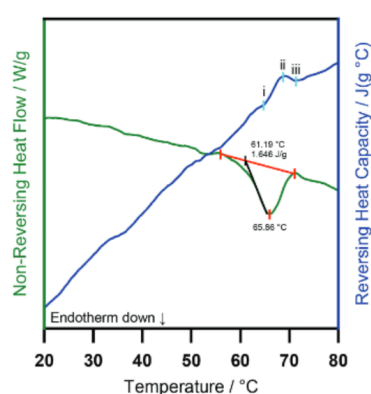
Project Management: Fritz Vollrath lead the overall project management while day-to-day project management was shared with the team.

This reporting period (covering yr 3, i.e. August 2017 to August 2018 has seen the coming together of a range of individual projects highly relevant to the overall goal. An overview of the different studies shall outline the different approaches taken and insights gained. All work covered here has been published and is referenced below, as well as being listed on www.oxfordsilkgroup.com where the papers can be directly downloaded.

This year's focus was following up on last year with the aim to gain a more solid understanding of the basic silk material what we de-construct into its molecular constituents using chaotropic agents such as LiBr. These liquid silk precursor dopes were then used (and tested for performance) in spinning rigs to form silk filaments. This makes the first section of the report, with the papers published embedded. In addition we used some of these liquid silks to cast composites such as horn (as 1st model) and ivory (as 2nd model), with the rhino horn work completed and the ivory work, which is much more challenging, still ongoing.

Section (A): Silk Dopes and Natural Filaments

To follow up on our investigation of the folding transitions of silk dopes using insights from synthetic biology we focused on last year we now completed a study on the thermal behavior of a range of silk dopes ranging from native in-gland silks to reconstituted silks. Importantly, native silk proteins, extracted directly from the silk gland prior to spinning, offer access to a naturally hydrated protein that has undergone little to no processing. Combined with differential scanning calorimetry (DSC), it is possible to probe the thermal stability and hydration status of silk and thus investigate its denaturation and solidification, echoing that of the natural spinning process. It is found that native silk is stable between $-10\text{ }^{\circ}\text{C}$ and $55\text{ }^{\circ}\text{C}$, and both the high-temperature enthalpy of denaturation (measured via modulated temperature DSC) and a newly reported low-temperature ice-melting transition may serve as useful quality indicators in the future for artificial silks. Finally, compared to albumin, silk's denaturation enthalpy is much lower than expected, which is interpreted within a recently proposed entropic desolvation framework which can serve to unveil the low-energy aquamelt processing pathway.



*MT-DSC thermogram of a representative native *B. mori* feed-stock sample.*

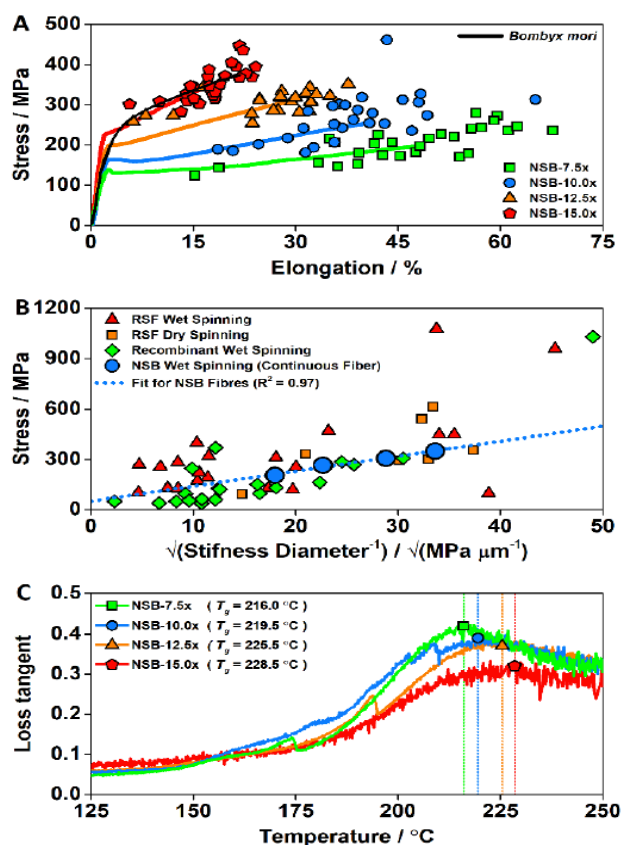
Non-reversing heat flow is shown in green and the underlying heat capacity is presented in blue. Changes in underlying heat capacity through the denaturation endotherm are labeled as i) onset of dehydration; ii) peak in heat capacity signal due to dehydration and structural reconfiguration; iii) return to heat capacity baseline once denaturation process is complete. Note that the exotherm is up.

Reference: 2018 Holland, C., Hawkins, N., Frydrych, M., Laity, P., Porter, D. & Vollrath, F. - Differential Scanning Calorimetry of Native Silk Feedstock. Macromol Biosci. 2019 Mar;19(3):e1800228. doi: 10.1002/mabi.201800228.

This data, in addition to the analytical tool of Differential Scanning Fluorimetry (DSF) allows us not to modify and tune our feedstocks for the subsequent spinning trials (see next paragraph on spinning). Importantly, this technique allowed us to probe and test not only the native silk itself, but also the effects of potential molecular folding disruptors and additives such as active dyes (see paragraph (B) on functionalisation).

Spinning from native dope: Spiders and silkworms spin their silks from aqueous protein feedstock using a self-assembly fibrillation process. This flow-induced phase-transition of the safely stored silk molecules is irreversible, environmentally clean and supremely energy efficient. The process seems to rely on the complex structure of the native silk protein molecules. Here we test this hypothesis by bio-spinning native silk feedstock, extracted in a dedicated process, into silk filaments that equal their natural models in mechanical

properties. Importantly, the filaments and fibers had the slender dimensions of native silk fibers as well as their hierarchical nanofibrillar structures. Bio-inspired modulation of spinning and post-draw conditions directly affected mechanical properties in correlation with fiber crystallinity, i.e. the degree of molecular, just as it does in native fibres. We believe that our study contributes significantly to the development of artificial silks by showing that the key to successful bio-spinning will be in the design of appropriate feedstock properties. This, in turn, will have significant implications for low energy synthetic polymer processing.



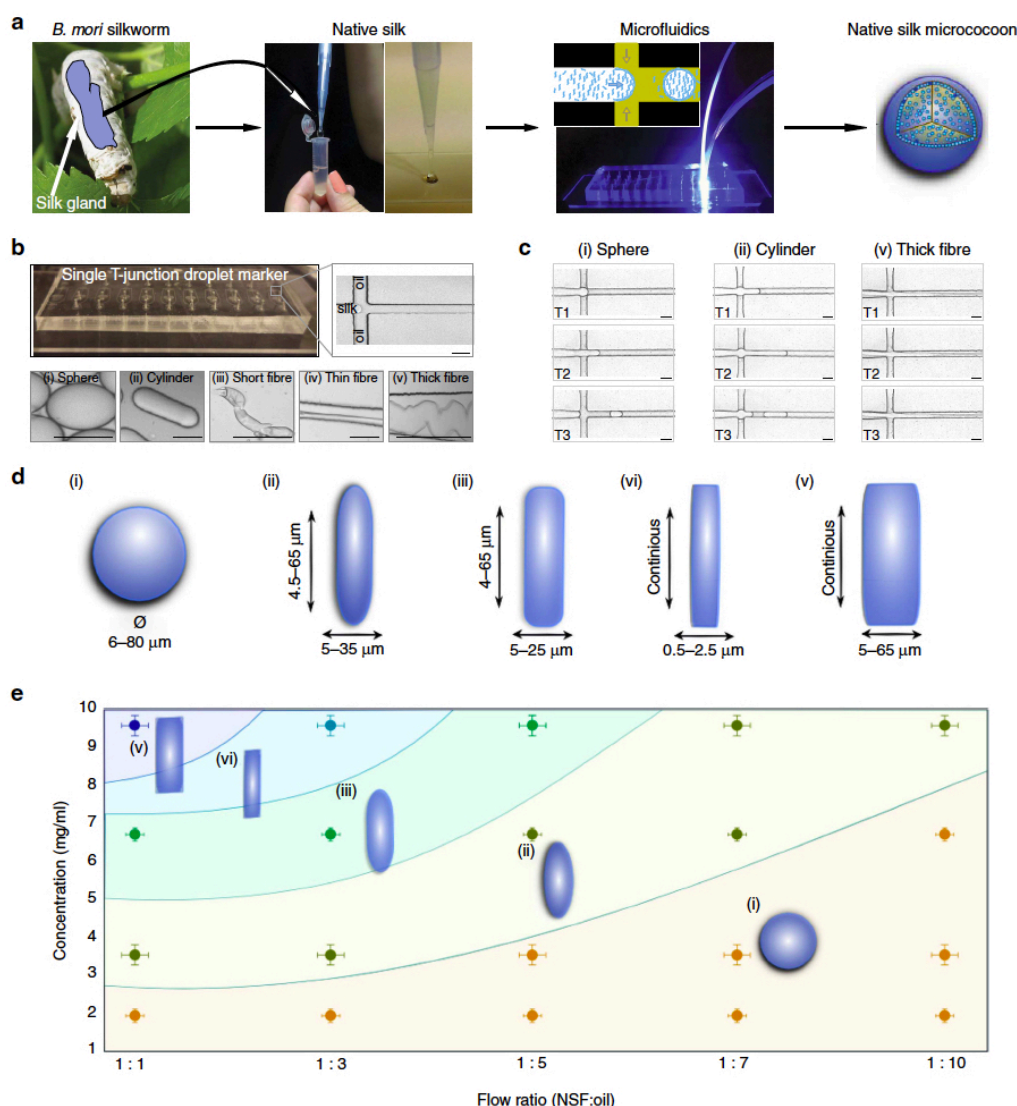
Mechanical properties of NSB fibers.

(A) Representative quasi-static tensile stress–strain curves of NSB fibers, fabricated at various draw-ratios and in comparison to natural silk fiber. (B) Comparison of the fracture-strength relationship of NSB fibers with various artificially spun silk fibers from literature. (C) Representative loss tangent-temperature curves of NSB fibers.

Reference: 2019 Frydrych M, Greenhalgh A & Vollrath F - Artificial spinning of Natural Silk threads. in review with Scientific Reports

In addition to spinning using a spider-inspired process, we also explored the use of microfluidics-based strategies to spin liquid native silk dope (obtained directly from the silk gland of *Bombyx mori* silkworms) into micron-scale capsules with controllable geometry and variable levels of intermolecular β -sheet content in their protein shells. We demonstrated that such micrococoon can store internally the otherwise highly unstable liquid native silk for several months and without apparent effect on its functionality. We further demonstrated that these native silk micrococoon enable the effective encapsulation and release of other aggregation-prone proteins, such as functional antibodies. These results show that native silk micrococoon are capable of preserving the full activity of sensitive cargo proteins that can aggregate and lose function under conditions of bulk storage, and thus represent an attractive class of

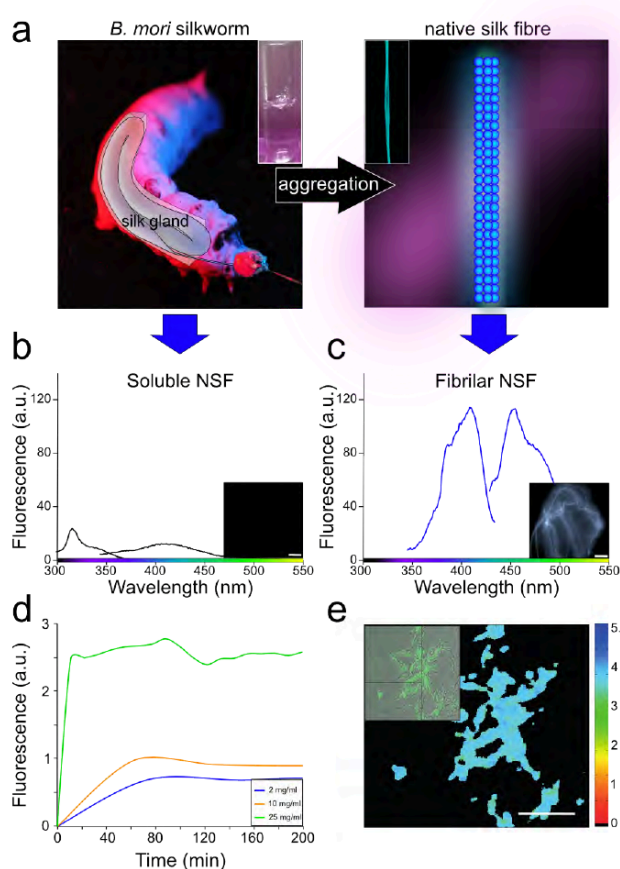
materials for the storage and release of active biomolecules.



The microfluidic manufacture of silk micro-cocoons: (a) Schematic representation of the microfluidic processing of NSF into micrococoons. (b) Optical microscopy images of the NSF micrococoons formed at a single T-junction in the microfluidic device. Micrographs of a variety of NSF micrococoon shapes are shown in the lower panels: (i) sphere, (ii) cylinder, (iii) short fibre, (iv) thin fibre, (v) thick fibre. Scale bar, 20 μm . (c) Micrographs of NSF micrococoon formation acquired at three different time points T1, T2 and T3: (i) sphere; T11/40 ms, T21/410 ms, T31/413 ms, (ii) cylinder; T11/40 ms, T21/413 ms, T31/434 ms and (v) thick fibre; T11/40 ms, T21/459 ms, T31/468 ms. Scale bar, 20 μm . (d) A schematic representation showing the characteristic dimensions of the NSF micrococoons generated in this study. (e) Different micrococoon shapes generated as a function of the protein concentration and of the ratio of the flow rates of the aqueous to oil phases.

Reference: 2017 Shimanovich, U., Ruggeri, S.F., De Genst, E., Adamcik, J., Barros, T.P., Porter, D., Muller, T., Mezzenga, R., Dobson, C.M., Vollrath, F., Holland, C. & Knowles, T.P.J. - Silk micrococoons for protein stabilisation and molecular encapsulation Nature Comms, 8: 15902

Key characteristics of native silk dopes are directly associated with the physical transformation of unstructured, soluble NSF, into highly organised nano- and microscale fibrils rich in β -sheet content. We were able to show that this NSF fibrillation process is accompanied by the development of intrinsic fluorescence in the visible range, upon near-UV excitation, a phenomenon that has not been investigated in detail to date. We probed the optical and fluorescence characteristics of NSF fibrils and explored their potential applications for the development of self-assembled optically active biomaterials and systems. In particular, the multi-disciplinary and multi-university demonstrated that NSF can be structured into auto-fluorescent microcapsules with a controllable level of β -sheet content. We were able to show, furthermore, a facile and efficient fabrication route that permits arbitrary patterns of NSF microcapsules to be deposited on substrates under ambient conditions. The resulting fluorescent NSF patterns display a high level of photostability. These results demonstrate the potential of using native silk as a new class of biocompatible photonic material.



Biophotonics of Native Silk

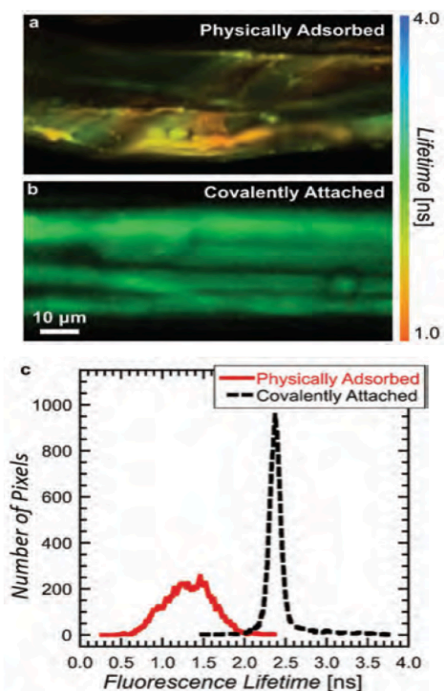
(a) Image of *B. mori* silkworm (left) and schematic representation of a native silk fibril (right). The insets show images of soluble NSF which was extracted directly from the *B. mori* silkworm gland (left) and aggregated NSF on the right, exhibiting its fibrillar nature. (b) Fluorescence spectra of soluble and (c) aggregated NSF. The latter exhibits distinct excitation and emission peaks at 405 nm and 450 nm, respectively. The corresponding fluorescence microscopy images are shown as insets. Scale bars = 5 μ m. (d) Evolution of the intrinsic NSF fluorescence as a function of aggregation time and protein concentration. (e) Inset: Overlay of differential interference contrast (DIC) and intrinsic fluorescence images of NSF aggregates in bulk. Main panel: Fluorescence lifetime image for the same field of view. Images were obtained with a confocal microscope with TCSPC capability using a laser excitation wavelength of 405 nm and a detection window of 50 nm centred at 475 nm. Scale bar = 10 μ m.

Reference 2018 Shimanovich, U., Pinotsi, D., Shimanovich, K., Yu, N., Bolisetty, S., Adamcik, J., Mezzenga, R., Charmet, J., Vollrath, F. & Gazit, E. - *Biophotonics of Native Silk Fibrils*. *Macromolecular bioscience* 18: (4)

Section (B): Silk Composites

While silks have intrinsic fluorescence, we can also functionalise a silk for reactive composites using artificial fluorophores acting either as aquaphores or as mechanophores. To study the interfacial damage in a silk composite we used two-photon fluorescence life-time imaging microscopy (TP-FLIM), following

uniaxial tensile deformation of a silk fibroin epoxy composite. This was accomplished using a simple two-step process to incorporate a mechanoresponsive fluorescent probe at the interface, combined with the use of hyperspectral fluorescence imaging and TP-FLIM characterization. The spectral and life-time data confirmed the extent of covalent bonding of the probe at the interface. This measurement platform (multi-modal fluorescence microscopy coupled with mechano-responsive probes) shows potential as a powerful tool to accelerate the development of process-structure-property relations in composites.

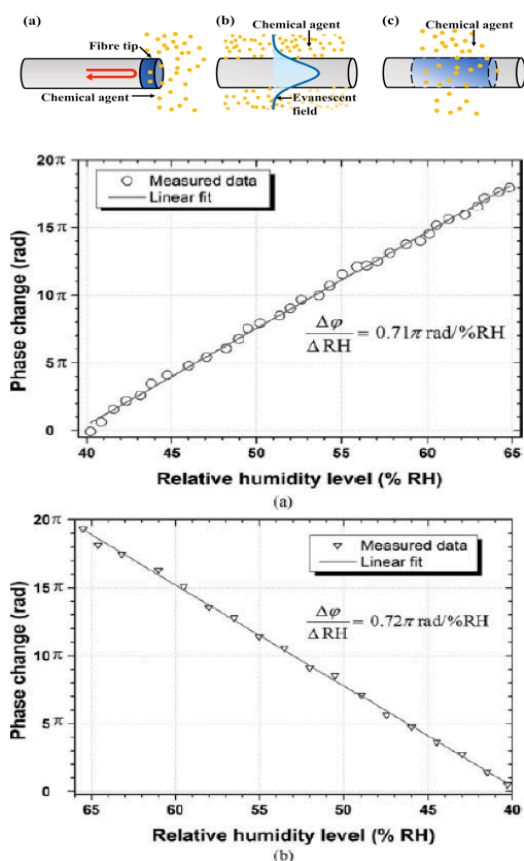


2P-FLIM analysis of physically and covalently labeled silk fiber bundle lifetime distributions.

FLT image of a) physically adsorbed and b) covalently attached MP on silk fiber bundle. The color scale to the right of the images indicates FLT values and applies to both images. c) Comparison of average FLT distributions for physically adsorbed and covalently. The covalently attached dye shows a narrower FLT distribution and a longer average lifetime.

Reference: 2017 Woodcock, J.W., Beams, R., Davis, S.D., Chen, N., Stranick, S.J., Shah, D.U., Vollrath, F. & Gilman, J.W. - Observation of Interfacial Damage in a Silk-Epoxy Composite, Using a Simple Mechanoresponsive Fluorescent Probe *Advanced Materials Interfaces* 4:10

Importantly, we can potentially use functionalised silks as probes integrated into a fire-optic system. We used the wave-guiding properties of native dragline silk in a pioneering proof-of-concept experiment by spicing spider silk as an optical fiber to measure humidity content.



Integrating Native Spider Silk into an Optical Fiber for Humidity Sensing

Top Image: Typical fibre-optic chemical sensors based on (a) fibre tip and (b) evanescent field sensing. (c) Simple transmission setup, based on a chemically reactive fibre showing our set-up.

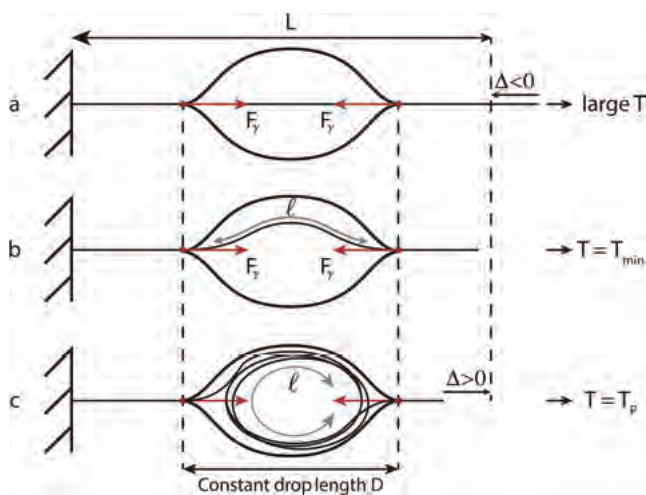
Lower Images: Measured phase change when the relative humidity level is (a) increased from 40 to 65% and (b) reduced from 65 to 40% at a temperature of 24 °C.

Reference: 2018 Hey Tow, K., Chow, D., Vollrath, F., Dicaire, I., Gheysens, T. & Thévenaz, L. - Exploring the Use of Native Spider Silk as an Optical Fiber for Chemical Sensing. J. Lightwave Technol. 36: (4) 1138-1144

Last but not least, during this reporting period we finalised a key study on the physics of the orb web spider's in-drop reeling mechanism. Here we examined further the mechanics of elastic fibres carrying liquid droplets. In such systems, buckling may localize inside the drop cavity if the fibre is thin enough. This so-called drop-on-coilable-fibre system exhibits a surprising liquid-like response under compression and a solid-like response under tension. Here we analyze this unconventional behavior in further detail and find theoretical, numerical and experimental evidence of negative stiffness events. We find that the first and main negative stiffness regime owes its existence to the transfer of capillary-stored energy into mechanical curvature energy. The following negative stiffness events are associated with changes in the coiling morphology of the fibre. Eventually coiling becomes tightly locked into an ordered phase where liquid and solid deformations coexist.

In conclusion of the overall in-drop-coiling project (which stretched over several years) we were able to show that the possibility of transferring wetting energy into mechanical energy leads to events of negative stiffness regimes in drop-on-coilable-fibre systems. The consequences include force undershoots in displacement-controlled setups and mechanical hysteresis in force-controlled setups. In both cases, quantitative agreement between experiments, theory and

numerics has been reached. We observed that further occurrences of negative stiffness regimes are linked to changes in the coiling morphology, and showed that a lock-in transition may eventually occur, underlining the link between the in-drop fibre length and the drop deformation. Drop-on-coilable-fibre systems thus open new possibilities as complex actuators in light of their unconventional mechanical response. For instance, accurate control may yield new routes to 3D microfabrication and reconfigurable coil-to-cage devices in liquid environments.



The subcritical nature of the coiling mechanism derives from the specific mechanics of in-drop capillary buckling.

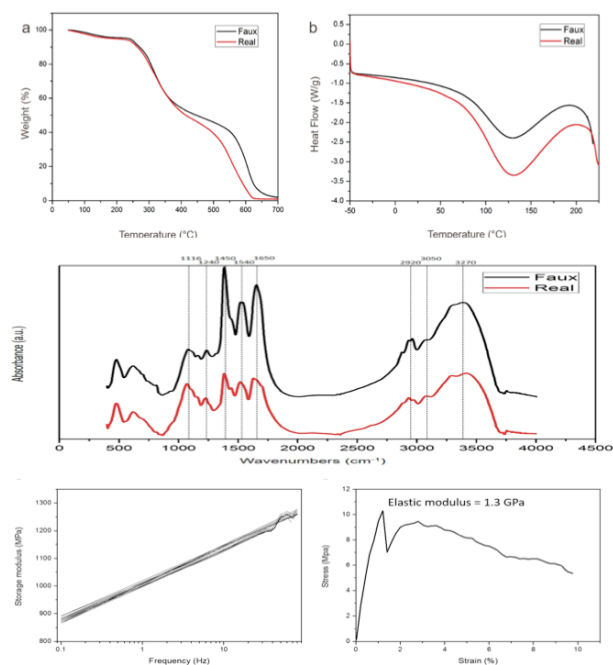
(a) The fibre is under large tension, preventing it from coiling locally. (b) The tension reaches a minimum T_{\min} and buckling begins. The drop retains its size D , while swallowing the fibre. (c) The in-drop fibre length c over which coiling occurs increases from D (in case (a)) to $D + D$ (in cases (b) and (c)), which decreases the system resistance

Reference: 2017 Elettro, H., Neukirch, S., Vollrath, F. & Antkowiak, A. - The mechanisms by which Drop-on-coilable-fibre systems exhibit negative stiffness events and transitions in coiling morphology. *Soft Matter* 13, 5509-5517

Section (C): Rhino Horn

Our investigation of making faux rhino horn demonstrated that it is rather easy as well as inexpensive to make a bio-inspired horn-like material that mimics the rhino's extravagantly expensive tuft of nose hair. We leave it to others to attempt to take our technology further and perhaps even so far as to fool punters into buying it in replacement or even preference to the real, and outrageously expensive, rhino horn. Whether flooding the market with confusing horn copies will ultimately lead to saving rhinos roaming in the wild remains to be seen. We can only hope because we, in our ignorance as material biologists not conservation economists, presently see little practical alternative to driving down the price in parallel to diligent guarding in the wild the real material and its pachyderm carriers. However that opinion may be taken, our artificial silk & horsetail horn mimic did rather well in our comparative analyses. Optical and Scanning Electron microscopy showed similar outer appearance and inner structure between real and artificial materials. Thermal analysis of both horns showed comparable thermal stability. FTIR showed no obvious differences between the natural and artificial horn. Mechanical Testing, finally, confirmed that the artificial copy behaved similar to the natural original. Together, our results demonstrated that the composite of horse tail-hair embedded in a silk

fibroin matrix can provide a fine biomimetic replacement for real rhinoceros horn. Besides, the structure of the rhino horn, as a fibre reinforced composite material, the fibres provide greater tensile strength than does the matrix, while the matrix provides greater ductility than the fibres.



Thermal, spectral and Mechanical testing of the real rhino horns and artificial horns.

The TGA tests (a), samples were heated in air from 20 °C to 800 °C with the speed of 10 °C/min. (a) Differential Scanning Calorimetry (DSC) (b) The Thermal Gravimetric Analysis (TGA) (c) The Fourier Transform Infra-Red (FTIR) Spectroscopy with Dynamic Mechanical Thermal Analysis (DMTA) in a Frequency sweep (d) and in a Strain sweep (e)

Reference: Mi R, , Shao ZZ and Vollrath F - A relative's hair makes for superb artificial rhino horn. MS in preparation

Other work, largely collaborative using our expertise and equipment, during this reporting period examined :

- *The Toughness-enhancing metastructure in the recluse spider's looped ribbon silk.* (2017 Koebley, S.R., Vollrath, F. & Schniepp, H.C. - *Mater. Horiz.*, 2017, 4: 377-382)

- *A comparison of the microstructure and mechanical properties of Bombyx mori and Antheraea pernyi cocoon composites* (2017 Guan, J., Zhu, W., Liu, B., Yang, K., Vollrath, F. & Xu, J. - *Acta Biomaterialia*, 47: 60-70)

- *Chemical relationships of ambers using attenuated total reflectance Fourier transform infrared spectroscopy* (2017 Cotton, L.J., Vollrath, F., Brasier, M.D. & Dicko, C. - *Geo. Soc. London, Special Publications*, 448)

- *An analysis of the structure and glass transition behaviour of silks for archaeology and conservation* (2018 Wang, J., Guan, J., Hawkins, N. & Vollrath, F. - *Journal of the Royal Society, Interface* 15: (139)

Research publications on work done throughout this 3yr award

- (1) Vollrath, F. 2016 The complexity of silk under the spotlight of synthetic biology *Biochemical Society Transactions*, 44 (4): 1151-1157
- (2) Dicko, C., Kasoju, N., Hawkins, N., & Vollrath, F. 2016 Differential scanning fluorimetry illuminates silk feedstock stability and processability, *Soft Matter*, 12: 255-262
- (3) Kasoju, N., Hawkins, N., Pop-Georgievski, O., Kubies, D. & Vollrath, F. 2016 Silk fibroin gelation via non-solvent induced phase separation *Biomater. Sci.*, 4: 460-473
- (4) Boulet-Audet, M., Holland, C., Gheysens, T. & Vollrath, F. 2016 Dry-Spun Silk Produces Native-Like Fibroin Solutions *Biomacromolecules*, 17 (10): 3198–3204
- (5) Shimanovich, U., Ruggeri, S.F., De Genst, E., Adamcik, J., Barros, T.P., Porter, D., Muller, T., Mezzenga, R., Dobson, C.M., Vollrath, F., Holland, C. & Knowles, T.P.J. 2017 Silk micrococoon for protein stabilisation and molecular encapsulation *Nature Comms*, 8: 15902
- (6) Kraznov, I., Seydel, T., Greving, I., Blankenburg, M., Müller, M. & Vollrath, F. 2016 Strain-dependent fractional molecular diffusion in humid spider silk fibres, *J. R. Soc. Interface*, 13 (122)
- (7) Woodcock, J.W., Beams, R., Davis, S.D., Chen, N., Stranick, S.J., Shah, D.U., Vollrath, F. & Gilman, J.W. 2017 Observation of Interfacial Damage in a Silk-Epoxy Composite, Using a Simple Mechanoresponsive Fluorescent Probe *Advanced Materials Interfaces* 4:10 (Collaboration with AFOSR NMSE partner)
- (8) Smitthipong, W., Suethao, S., Shah, D. & Vollrath, F. 2016 Interesting green elastomeric composites: Silk textile reinforced natural rubber *Polymer Testing*, 55: 17–24
- (9) Elettro, H., Neukirch, S., Vollrath F. & Antkowiak, A. 2016 In-drop capillary spooling of spider capture thread inspires hybrid fibers with mixed solid–liquid mechanical properties. *PNAS*, 113 (22): 6143-47 (10) Elettro, H., Neukirch, S., Vollrath, F. & Antkowiak, A. 2017 Drop-on-coilable-fibre systems exhibit negative stiffness events and transitions in coiling morphology. *Condensed Matter* 47: 60-70
- (11) Hey Tow, K., Chow, D., Vollrath, F., Dicaire, I., Gheysens, T. & Thévenaz, L. 2016 Weaving our way towards a new generation of fibre-optic chemical sensors based on spider silk *EPFL Conference paper*
- (12) Monks, J.N., Yan, B., Hawkins, N., Vollrath, F. & Wang, Z. 2016 Spider Silk: Mother Nature's Bio-Superlens *Nano Lett*, 16: 5842–5845
- (13) Mortimer, B., Soler, A., Siviour, C. R., Zaera, R. and Vollrath, F. 2016 Tuning the instrument: sonic properties in the spider's web, *J. R. Soc. Interface*, 13 (22)
- (14) Guan, J., Wang, Y., Mortimer, B., Holland, C., Shao, Z., Porter, D. and Vollrath, F. - Glass transitions in native silk fibers studied by DMTA. *Soft Matter*, 12, pp. 5926-5936
- (15) Guan, J., Zhu, W., Liu, B., Yang, K., Vollrath, F. & Xu, J. 2017 Comparing the microstructure and mechanical properties of Bombyx mori and Antheraea pernyi cocoon composites *Acta Biomaterialia*, 47: 60-70
- (16) Offord, C., Vollrath, F. & Holland, C. 2016 Environmental effects on the construction and physical properties of Bombyx mori cocoons *Journal of Materials Science* 51 (24): 10863–10872
- (17) Koebley, S.R., Vollrath, F. & Schniepp, H.C. 2017 Toughness-enhancing metastructure in the recluse spider's looped ribbon silk. *Mater. Horiz.* 4: 377-382
- (18) Shimanovich, U., Ruggeri, S.F., De Genst, E., Adamcik, J., Barros, T.P., Porter, D., Muller, T., Mezzenga, R., Dobson, C.M., Vollrath, F., Holland, C. & Knowles, T.P.J. 2017 Silk micrococoon for protein stabilisation and molecular encapsulation *Nature Comms*, 8: 15902
- (19) Cotton, L.J., Vollrath, F., Brasier, M.D. & Dicko, C. 2017 Chemical relationships of

- ambers using attenuated total reflectance Fourier transform infrared spectroscopy *Geo. Soc. London, Special Publications*, 448
- (20) Woodcock, J.W., Beams, R., Davis, S.D., Chen, N., Stranick, S.J., Shah, D.U., Vollrath, F. & Gilman, J.W. 2017 Observation of Interfacial Damage in a Silk-Epoxy Composite, Using a Simple Mechanoresponsive Fluorescent Probe *Advanced Materials Interfaces* 4:10
- (22) Elettro, H., Neukirch, S., Vollrath, F. & Antkowiak, A. 2017 Drop-on-coilable-fibre systems exhibit negative stiffness events and transitions in coiling morphology. *Soft Matter*
- (23) Guan, J., Zhu, W., Liu, B., Yang, K., Vollrath, F. & Xu, J. 2017 Comparing the microstructure and mechanical properties of Bombyx mori and Antheraea pernyi cocoon composites *Acta Biomaterialia*, 47: 60-70
- (24) Holland, C., Hawkins, N., Frydrych, M., Laity, P., Porter, D. & Vollrath, F. 2018 Differential Scanning Calorimetry of Native Silk Feedstock. *Macromol Biosci.*
- (25) Hey Tow, K., Chow, D., Vollrath, F., Dicaire, I., Gheysens, T. & Thévenaz, L. 2018 Exploring the Use of Native Spider Silk as an Optical Fiber for Chemical Sensing. *J. Lightwave Technol.* 36: (4) 1138-1144
- (26) Shimanovich, U., Pinotsi, D., Shimanovich, K., Yu, N., Bolisetty, S., Adamcik, J., Mezzenga, R., Charmet, J., Vollrath, F. & Gazit, E. 2018 Biophotonics of Native Silk Fibrils. *Macromolecular bioscience* 18: (4)
- (27) Wang, J., Guan, J., Hawkins, N. & Vollrath, F. 2018 Analysing the structure and glass transition behaviour of silks for archaeology and conservation. *Journal of the Royal Society, Interface* 15: (139)
- (28) Vollrath, F., Mi, R. & Shah, D.U. 2018 Ivory as an Important Model Bio-composite *Curator: The Museum Journal* 61 (1): 95-110

The following **Appendix** shows key components of the presentation given at the 2018 program review meeting as this presentation covered not only the 3rd year of work but it also reviewed all work done under this award over the full 3 year period in order to put the work into context.



UNIVERSITY OF
OXFORD

Silk: Transformations & Practical Uses

Fritz Vollrath

Department of Zoology
University of Oxford

AFOSR Program Review
Natural Material and Systems
Florida, USA
December 2018



Silk Transformations & Uses

Vollrath, Oxford

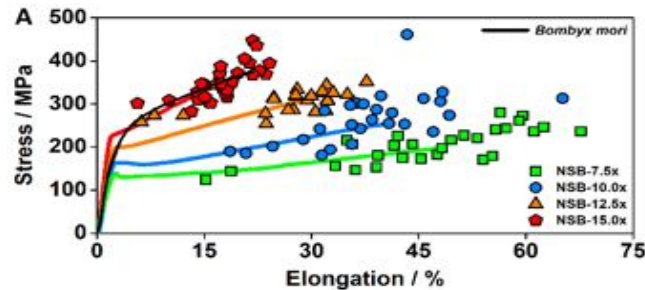


STATUS QUO

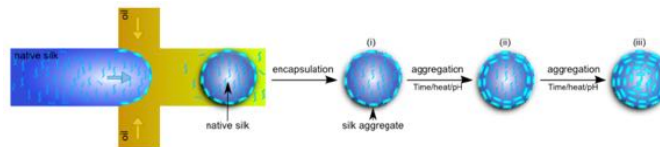
- (A) Properties of Natural and Reconstituted silk *feedstocks* still not well understood.
- (B) Silk transitions from melt to fibre barely studied.
- (C) Natural silk *spinning* requires elucidation.
- (D) Bio-inspired transition to novel materials lacking.

MAIN ACHIEVEMENTS:

- (a) Novel insights into silk transitions
- (b) Novel insights into dope properties
- (c) Spinning directly from such dopes



- (d) Successful microfluidic spinning of drops and filaments filled with long active material.



- (e) Insights into super tough 'silk' bio-composites such as faux ivory and rhino horn
- Other Achievements:** (a) many invited key-note lectures (b) summer exhibition at the Royal Society

QUANTITATIVE IMPACT

- (a) Up-take of Techniques
- (b) Scientific Citations
- (c) Translation to Practice
- (d) Transdisciplinary Collaborations



END-OF-PHASE GOAL

- (a) Better understand natural silk dope and related extrusion 'spinning' technology.
- (b) Overcome the differences between native and reconstituted silks.
- (c) Integrate natural silk composites into hybrid synthetic/silk composites.

NEW INSIGHTS

- (A) Production of fully functional Aquamelt silk fibroin dopes
- (B) Improved analytical techniques allow us to probe and test dope properties
- (C) Dope quality determines fibre properties and enables micro-fluidic manufacture.
- (D) Rhino Horn and Ivory provide model materials for novel silk composites, so do the spider capture silks.

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Program Goals

- **Understand the fundamentals of silk aquamelt-spinning technology i.e. the relationship between processing and structure that leads to good filaments by the process of cold extrusion with water as only solvent into a mild dehydrating bath followed by extensive draw-down.**
Example: successful spinning of silk filaments from native silk (taken from the animal) and from silk fibroin reconstituted from denatured silk filaments.
 - **Use insights from natural bio-composites to design and make functional copies.** Example: Elephant Ivory
- 1- Examine silk formation *in vitro*: dopes, flow, films and fibres
 - 2- Make filaments using insights gained from examining spiders and silk worms
 - 3- Make micro-structures using the inherent Aquamelt properties of the dope materials
 - 4- Expand understanding of diverse natural bio-composites to gain functional insights
 - 5- Make 3D composites that incorporate silks and examine their performance
 - 6- Study natural mineralised fibre-lay and fibre-scaffold systems as models
 - 7- Incorporate native silks into artificial composites and examine/tune properties.

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State of Progress

Overall Goal: Unravel the basic principles of silk dopes and fibers and the various relationships between temporal processing (of the dope) and final physical properties (of the filaments). Apply natural design characteristics of silk dopes and fibres to bio-spin analogues. Use silk dopes and filaments in the manufacture of bio-composites.

Progress to date:

- 1- Analysis of the 'quality' of natural silk dopes as judged by their transition properties
Transition criteria include analytical techniques such as Differential Scanning Fluorimetry (DSF) and Differential Scanning Calorimetry (DSC), Rheology and ultimately Aquamelt Extrusion Spinning into filaments with measurable mechanical properties
- 2- Empirical studies to explore Aquamelt dope storage and spinning technology with the ability of native silks and 'spiked' silk dopes to preserve their properties
Feeding nano-particles to spiders and doping extracted silkworm silks is leading to 'spiked' silks that have modified material properties. This approach allows us to design and make – for example - bespoke silk sensors where fluophores react to external conditions and indicate strains or hydration states.
- 3- Microfluidic manipulation of Aquamelt dopes into filled capsules and fibers.
natural silk dope and other highly reactive compounds stay 'alive' inside the silk shell
- 4- Understanding and copying morphology and mechanical properties of rhino horn and ivory.
First attempts promising with presentable rhino-horn and elephant ivory analogues

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Nature of Research: Scientific and Technological Transitions

Deployed established as well as devised novel analytical tools for the empirical examination of silk dopes and fibers.

Success in filament spinning trials testing the Aquamelt theory in practice.

Expand comparative studies of raw silk dope Aquamelts with derived biopolymer melts.

Dr Beth Mortimer (high rate performance of silks) hired as tenure track Faculty at Oxford

- Spin-out company to spin silk.
- Patent submitted, various patents under consideration



Publications 2017-18

- 1 2018 Holland, C., Hawkins, N., Frydrych, M., PLaity, P., Porter, D., Vollrath F. Differential Scanning Calorimetry of Native Silk Feedstock *Macromol. Bioscience*
- 2 2018 Mortimer, B., Soler, A., Siviour, C.R., & Vollrath, F. - Remote monitoring of vibrational information in spider webs. *The Science of Nature* 105: (37)
- 3 2018 Hey Tow, K., Chow, D., Vollrath, F., Dicaire, I., Gheysens, T. & Thévenaz, L. - Exploring the Use of Native Spider Silk as an Optical Fiber for Chemical Sensing. *J. Lightwave Technol.* 36: (4) 1138-1144 3)
- 4 2018 Shimanovich, U., Pinotsi, D., Shimanovich, K., Yu, N., Bolisetty, S., Adamcik, J., Mezzenga, R., Charmet, J., Vollrath, F. & Gazit, E. et al - Biophotonics of Native Silk Fibrils. *Macromolecular bioscience* 18: (4)
- 5 2018 Wang, J., Guan, J., Hawkins, N. & Vollrath, F. - Analysing the structure and glass transition behaviour of silks for archaeology and conservation. *Journal of the Royal Society, Interface* 15: (139)
- 6 2018 Vollrath, F., Mi, R. & Shah, D.U. - Ivory as an Important Model Bio-composite *Curator: The Museum Journal* 61 (1): 95-110
- 7 2017 Shimanovich, U., Ruggeri, S.F., De Genst, E., Adamcik, J., Barros, T.P., Porter, D., Muller, T., Mezzenga, R., Dobson, C.M., Vollrath, F., Holland, C. & Knowles, T.P.J. - Silk micrococoon for protein stabilisation and molecular encapsulation *Nature Comms*, 8: 15902
- 8 2017 Cotton, L.J., Vollrath, F., Brasier, M.D. & Dicko, C. - Chemical relationships of ambers using attenuated total reflectance Fourier transform infrared spectroscopy *Geo. Soc. London, Special Publications*, 448g
- 9 2017 Koebley, S.R., Vollrath, F. & Schniepp, H.C. - Toughness-enhancing metastructure in the recluse spider's looped ribbon silk. *Mater. Horiz.*, 2017, 4: 377-382
- 10 2017 Woodcock, J.W., Beams, R., Davis, S.D., Chen, N., Stranick, S.J., Shah, D.U., Vollrath, F. & Gilman, J.W. - Observation of Interfacial Damage in a Silk-Epoxy Composite, Using a Simple Mechanoresponsive Fluorescent Probe *Advanced Materials Interfaces* 4:10
- 11 2017 Elettro, H., Neukirch, S., Vollrath, F. & Antkowiak, A. - Drop-on-coilable-fibre systems exhibit negative stiffness events and transitions in coiling morphology. *Soft Matter*
- 12 2017 Guan, J., Zhu, W., Liu, B., Yang, K., Vollrath, F. & Xu, J. - Comparing the microstructure and mechanical properties of Bombyx mori and Antheraea pernyi cocoon composites *Acta Biomaterialia*, 47: 60-70

Stand at the Royal Society Summer Exhibition, by Invitation.

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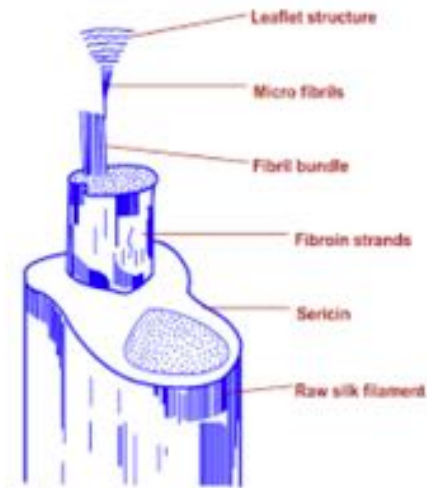
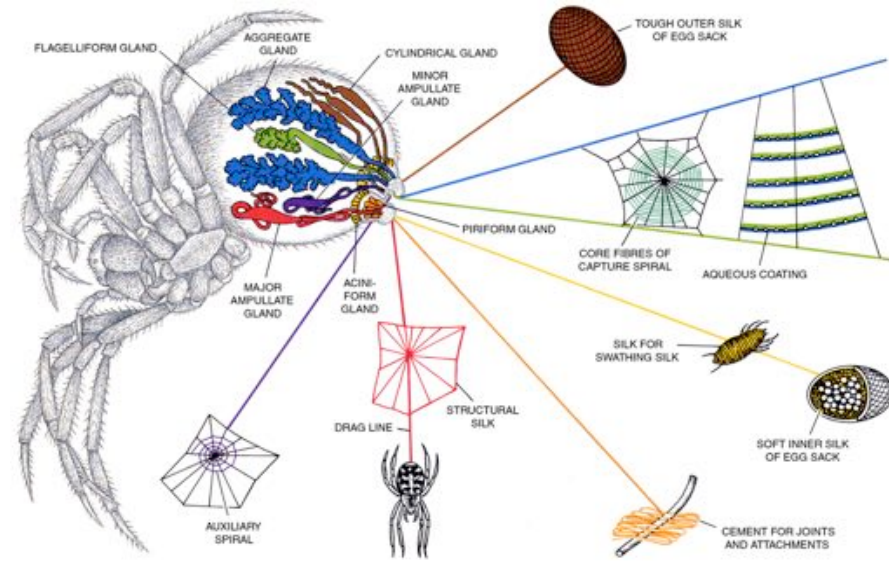


Transformational vs Evolutionary

- Our ***theoretical understanding*** of natural and reconstituted silk **melt dopes** is **transformational** (i.e. **generic and to be used by others**) as it provides novel and **revolutionary** novel insights into silks as a new class of self-assembling materials i.e. *Aquamelt* bio-polymers. This in turn could well open generic views of protein folding.
- Our unique ***combination of techniques*** coupled to a rare ***pool of equipment*** is also **evolutionary** by developing novel **in-house** technologies (e.g. DSF for proteins) and machines (e.g. Rheo-IR) while facilitating collaborations studying a wide range of biomaterials. But it also allows us to be **transformational** by developing novel **generic** understanding of silks.

What is SILK?

A multi-protein,
nano-scale
composite
that is
extrusion spun
to function
outside the body.



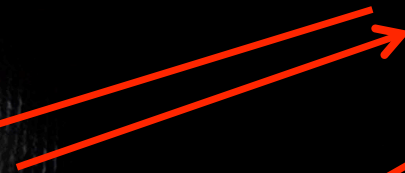
- 6x stronger than steel (weight for weight)
- 5x tougher than Kevlar
- 1000 times more energy efficient to produce
- chemical function & shape memory
- biodegradable & biocompatible

Outline of Talk

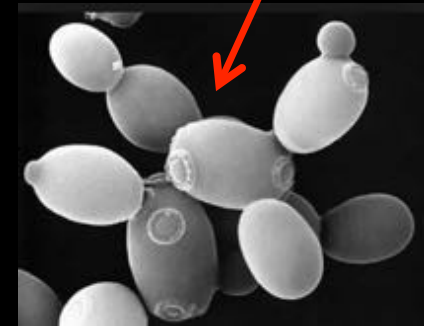
- Overview : put Silk in context
- Properties of Spinning Dope as Aquamelt
- Spinning Fibers from an Aquamelt
- Doping the Dope
- Microfluidic 'Spinning' and Encapsulation
- The Droplet as a silk-silk Composite
- Update on Silk Horn and Silk Ivory Work

What is and what is not a *Silk*

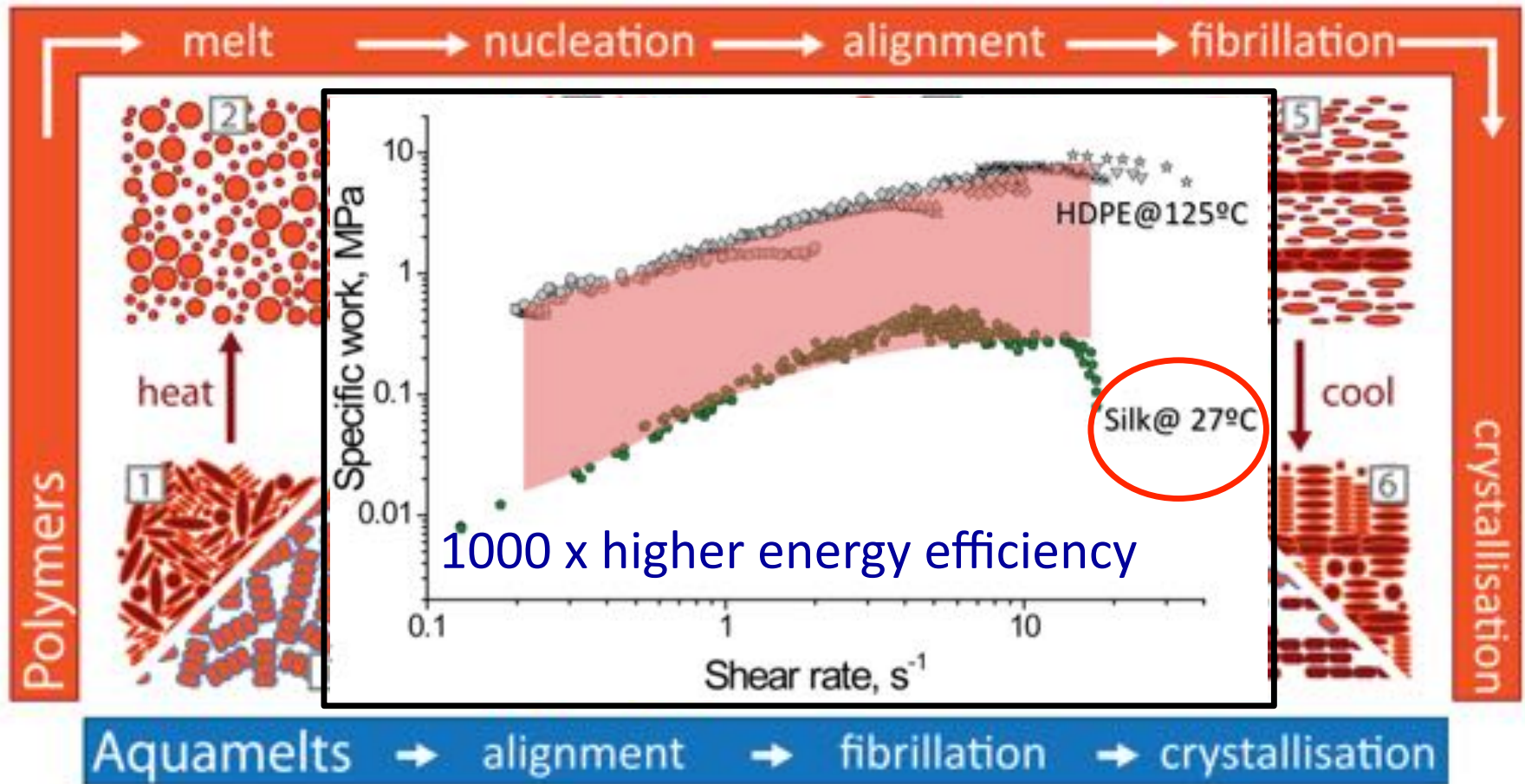
So far none of the GM dopes produced a natural silk - nor is any an *Aquamelt*.



Fake News !!



What is an Aquamelt?

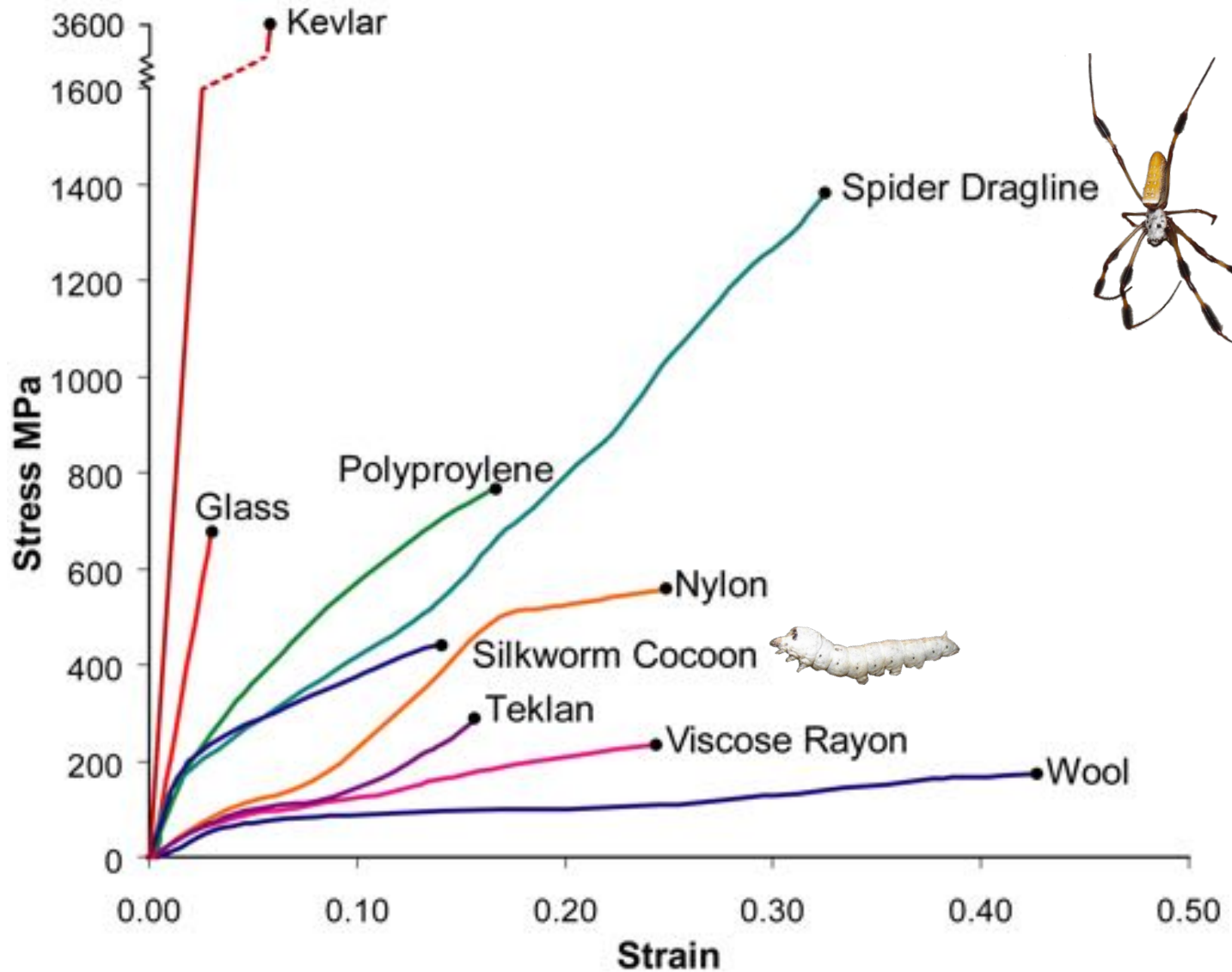


An aquamelt is a naturally hydrated polymeric material that is able to solidify at environmental temperatures through a controlled stress input

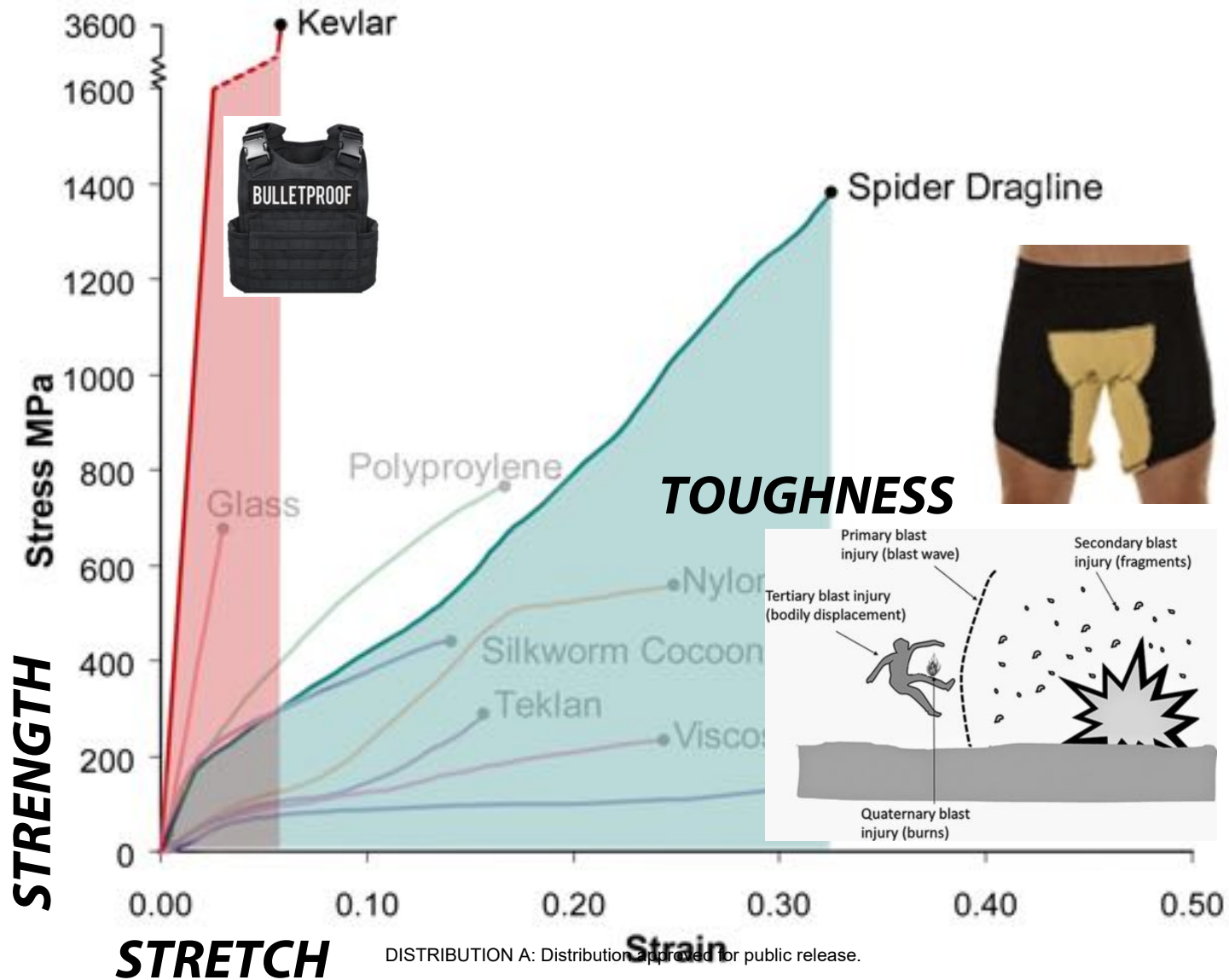
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(Holland *et al* (2012) *Advanced Materials*, 24 (1) 105-109, Vollrath *et al* (2013) *MRS Bulletin*

Silk Fiber Properties in Comparison

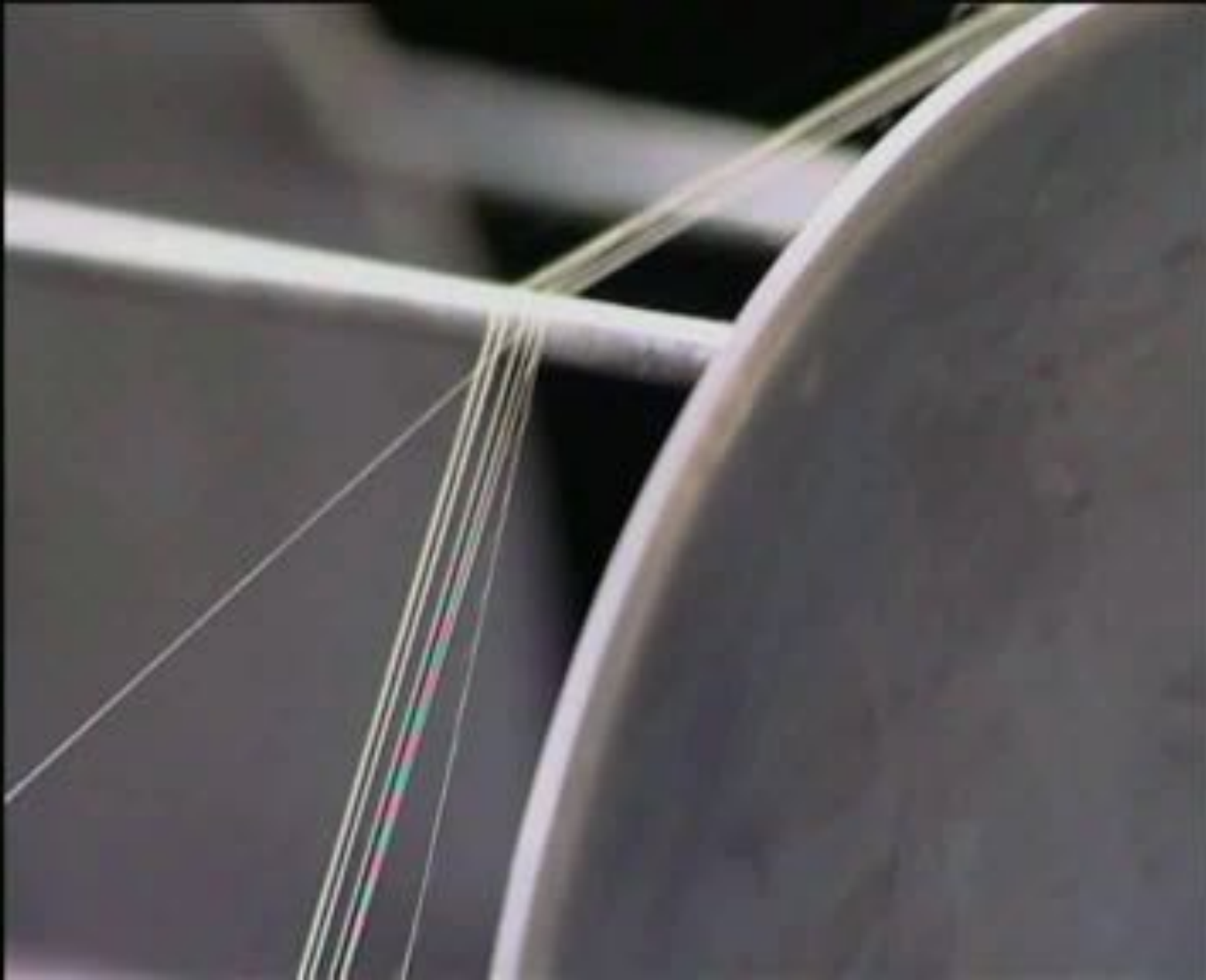


The Concept of Toughness



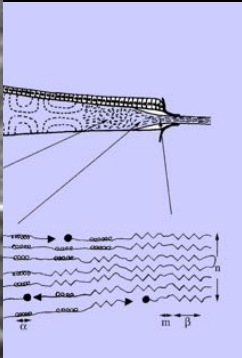
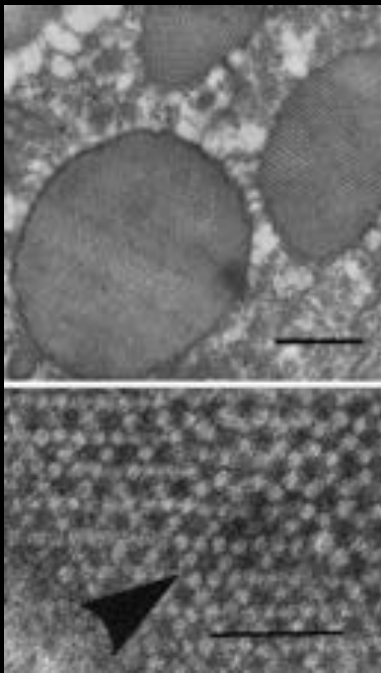
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Only spiders allow controlled 'silking'

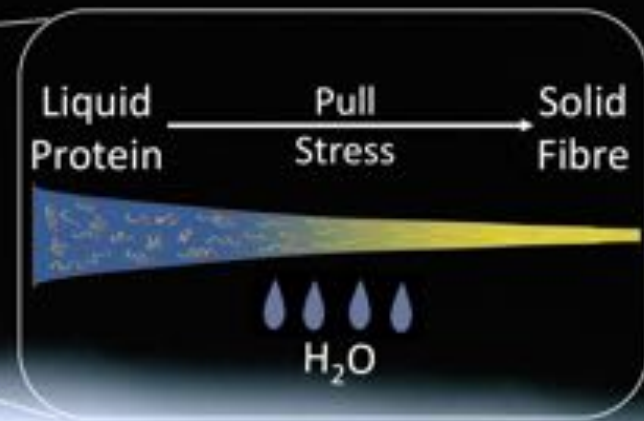
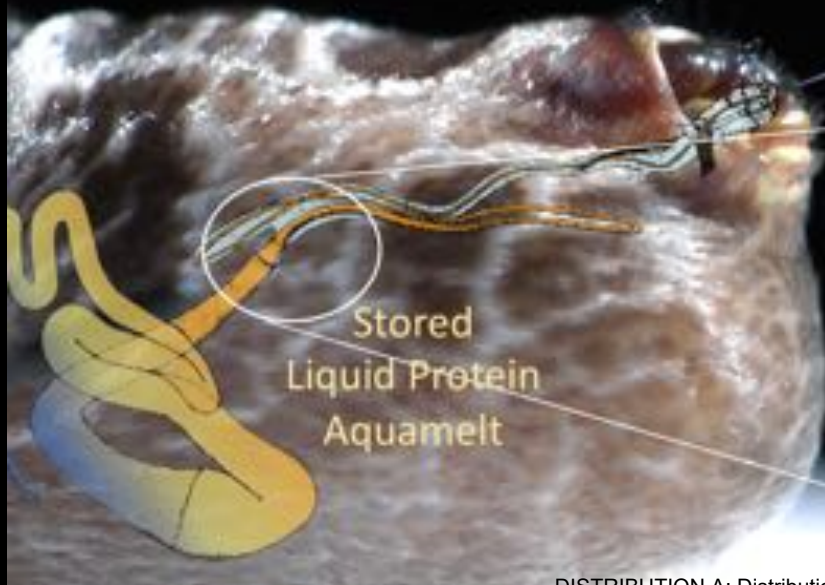


which allows extensive testing of research questions

The Animal's Aquamelt LC Spinning

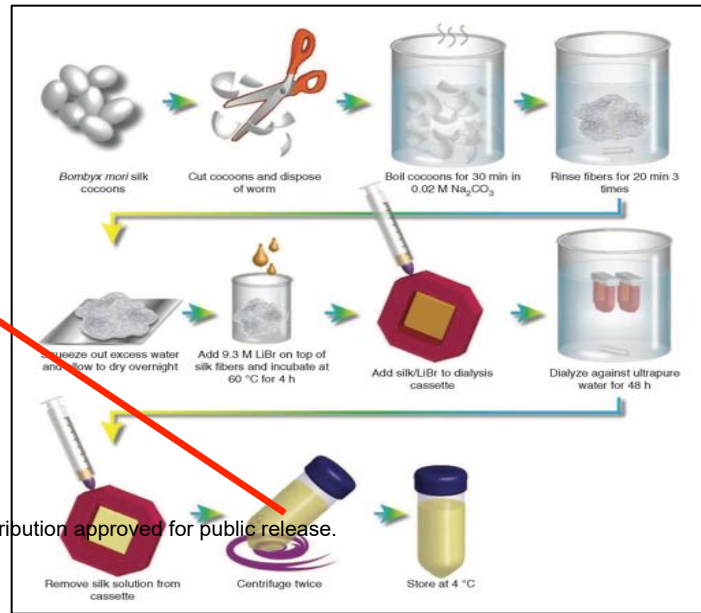
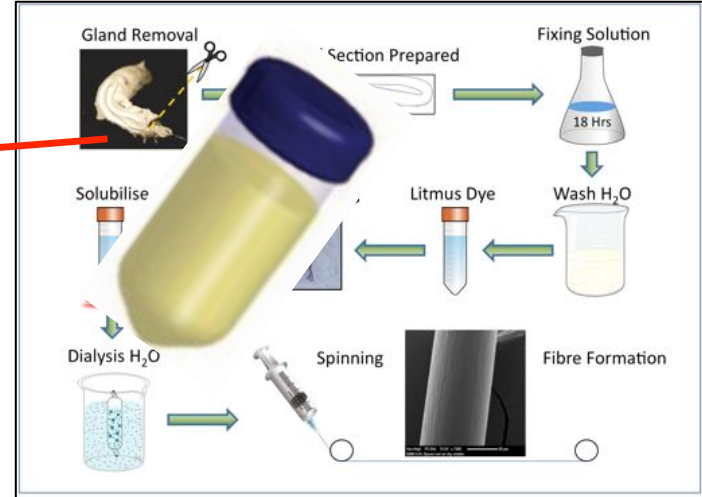
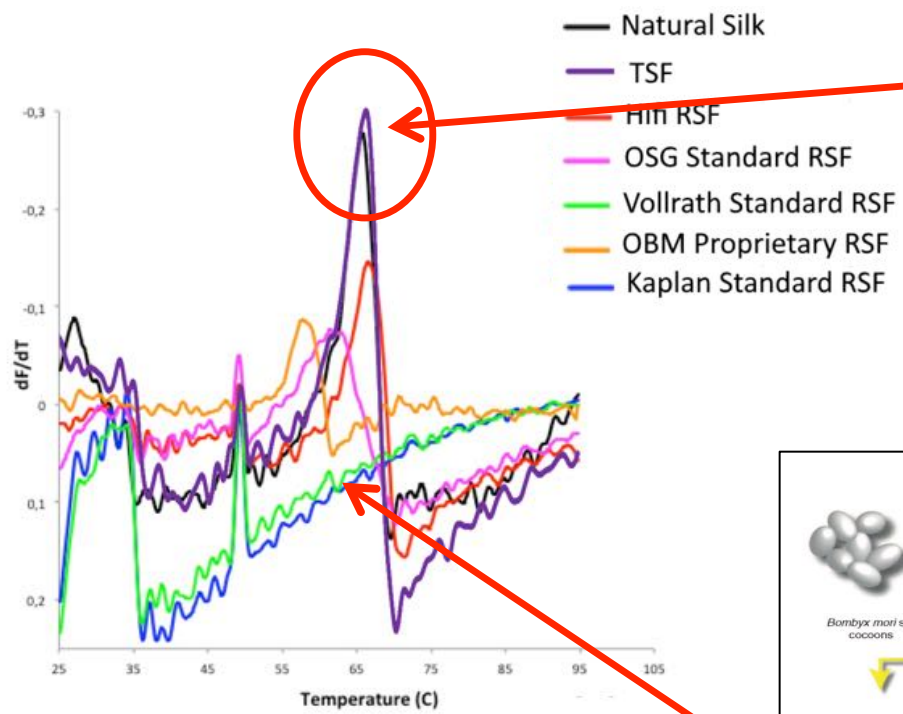
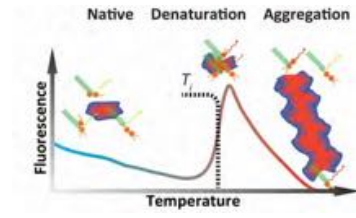


ENERGY INPUT :
Mechanical = flow elongation, shear
Chemical = pH, salting
Thermal = unlikely



The Dope is the Key to Aquamelt Spinning

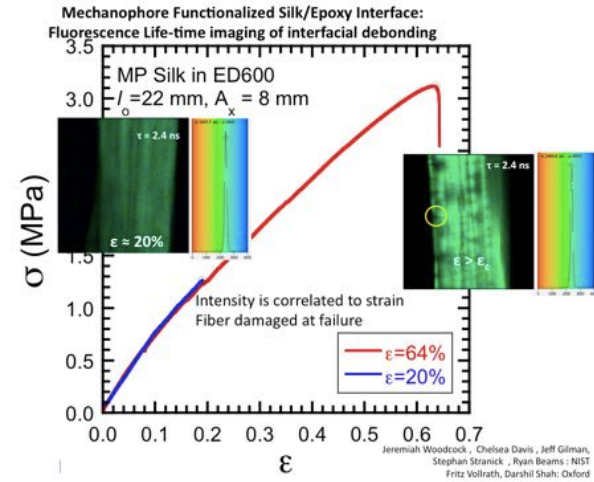
Comparing dope quality by monitoring 'denaturation' as function of temperature using Dynamic Scanning Fluorimetry in a real-time PCR



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Silk fibre/matrix interface analysis

- Collaborating with Jeff Gilman's group @ NIST
 Study the fibre/matrix interface at nano-, micro- and macro-level using **FRET and dyes/mechanophores**



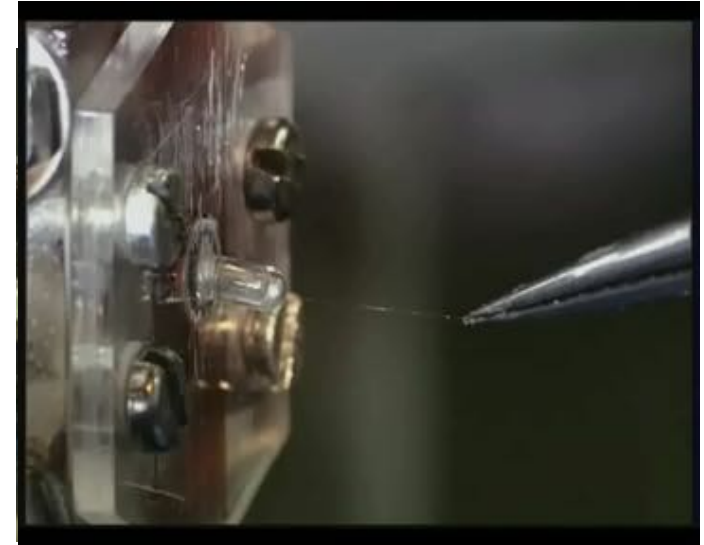
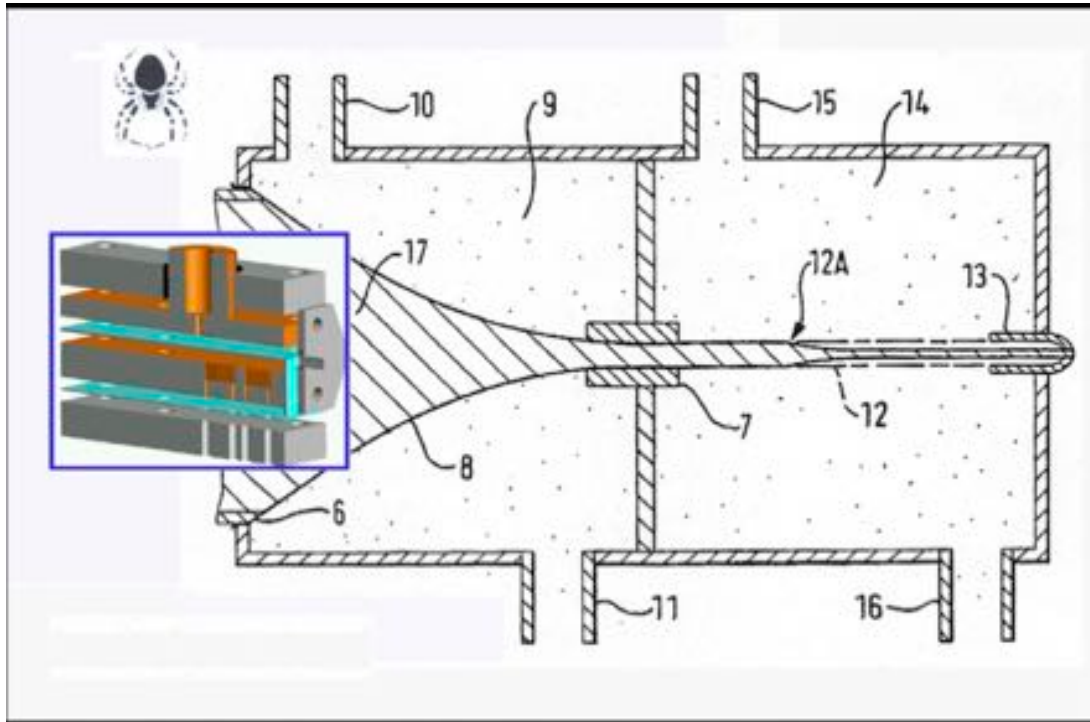
Mechanophore can be activated by light, water, temperature or strain/stress



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500 N tensile load on single-fibre silk/epoxy composites – mechanophore activates

Bio-inspired Applications



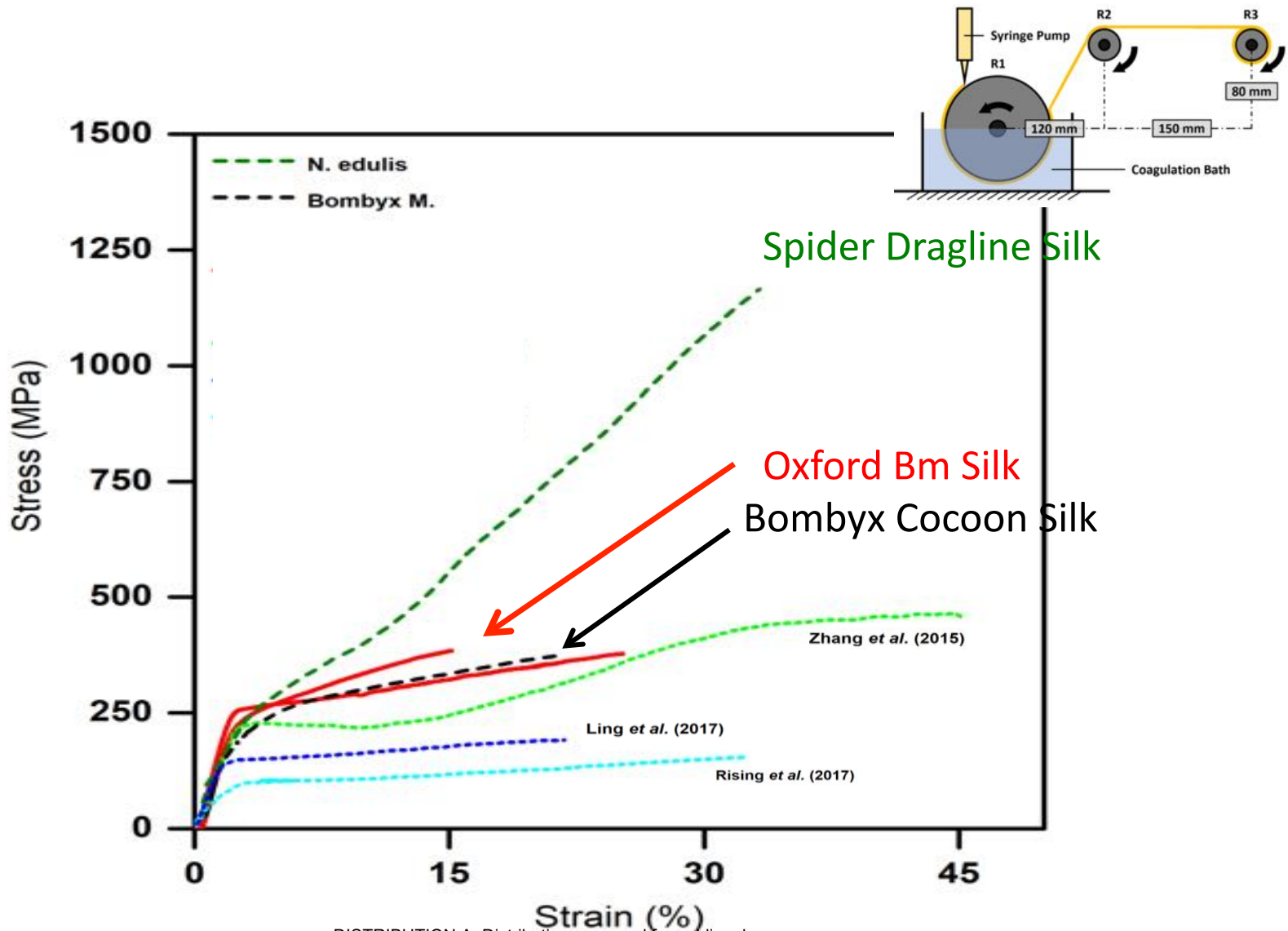
Spider Spinning
Technology

Using the Energy Efficiency of Natural Silk Spinning.

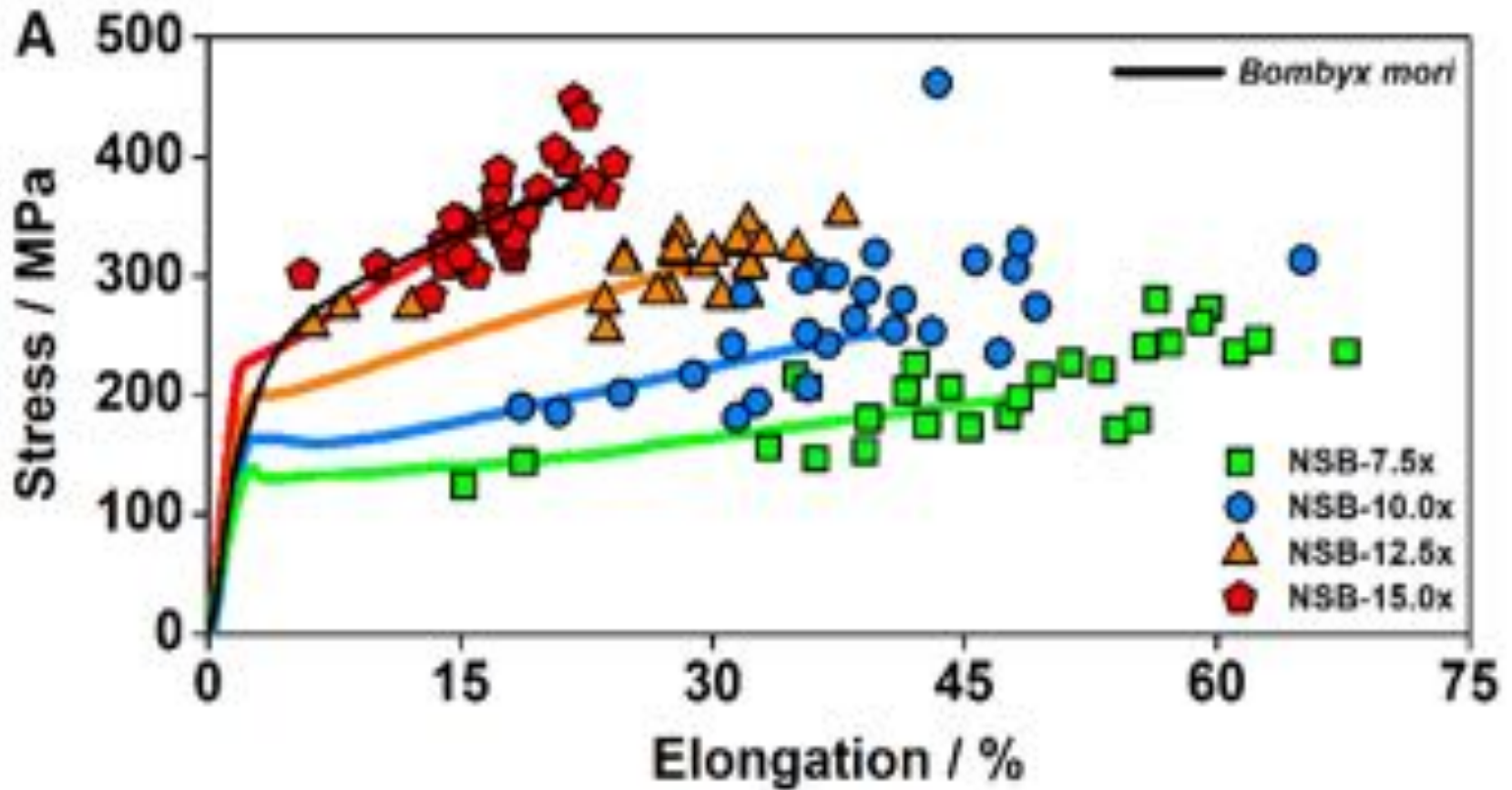
Exploring Nature's Aquamelt Bio-Spinning Technology.

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Spinning Aquamelt into real Silk Fibre

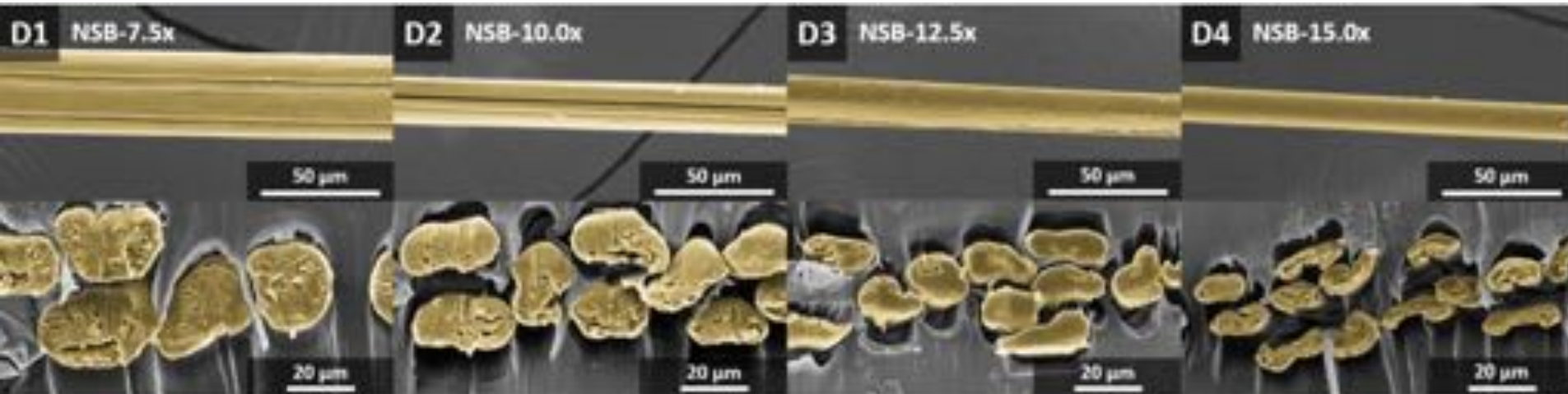
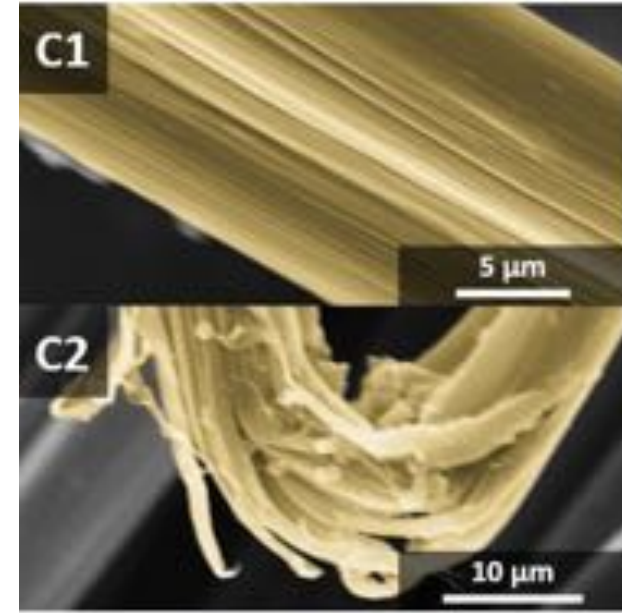
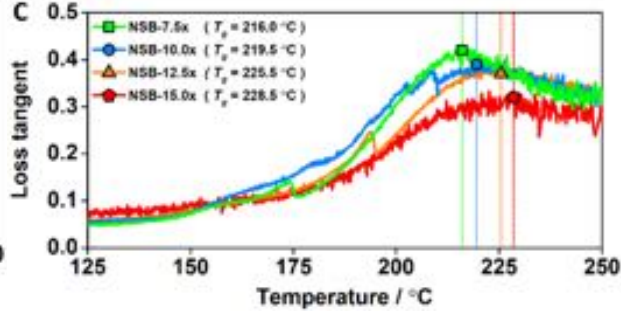
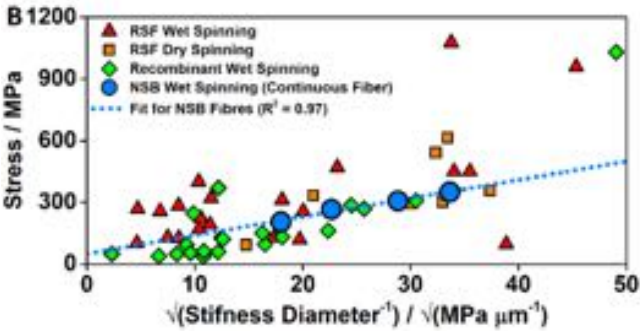
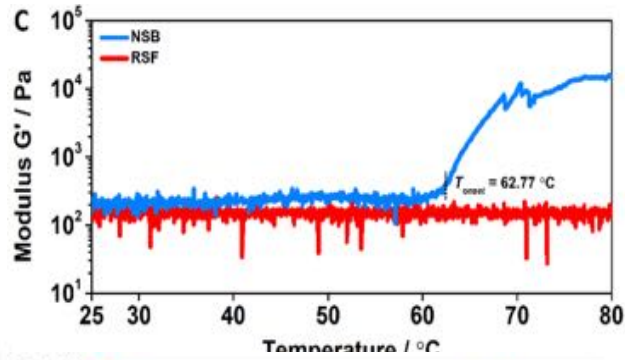
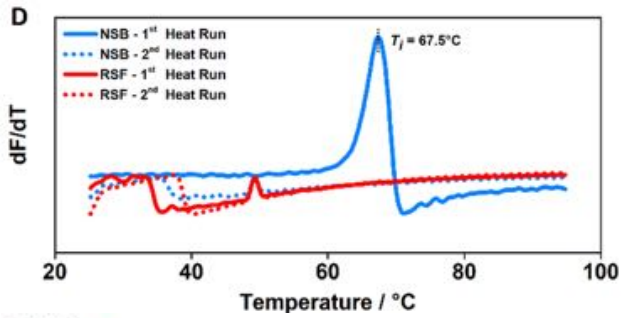


Tunability of the Oxford Bm-dope Silk



Achieved simply by varying spinning speeds and post-draw.

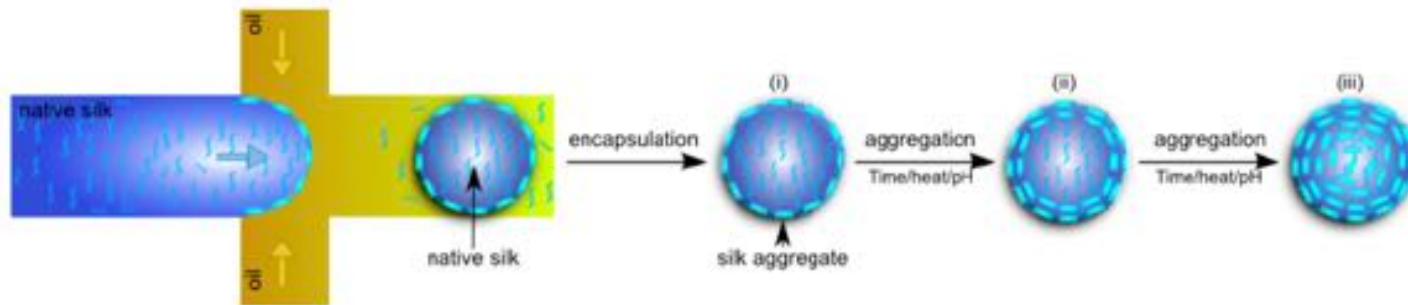
Aquamelt Spinning : Data

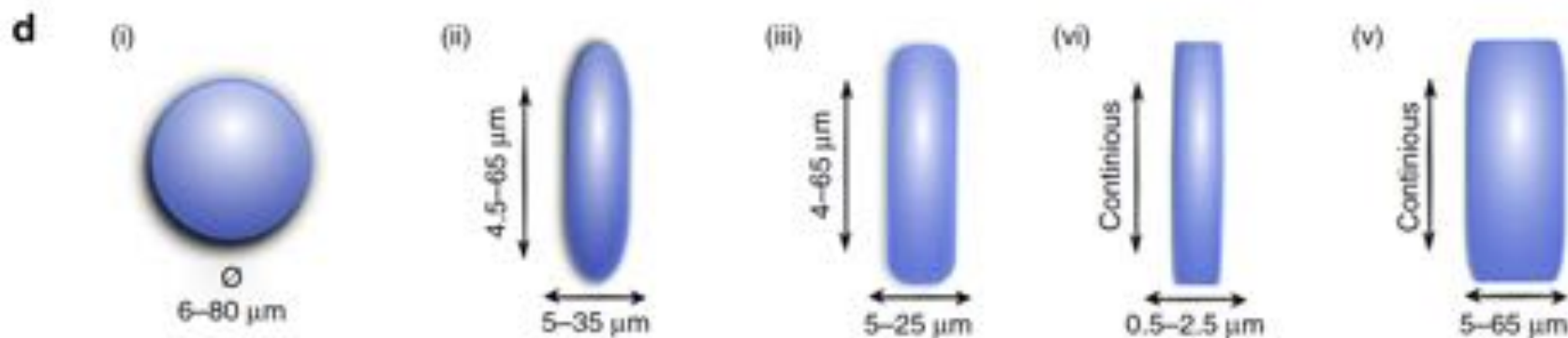
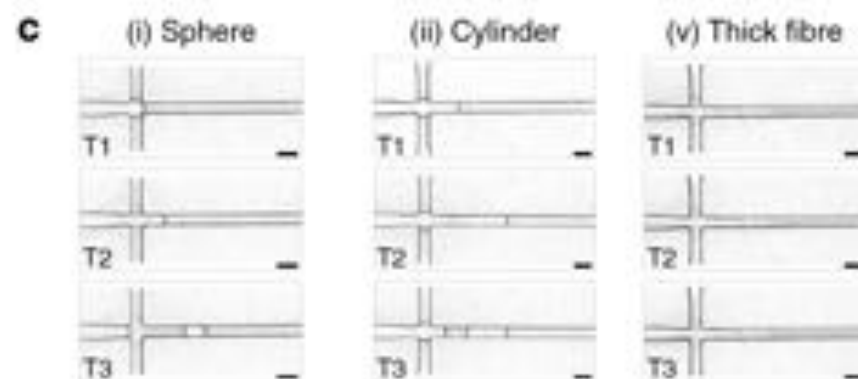
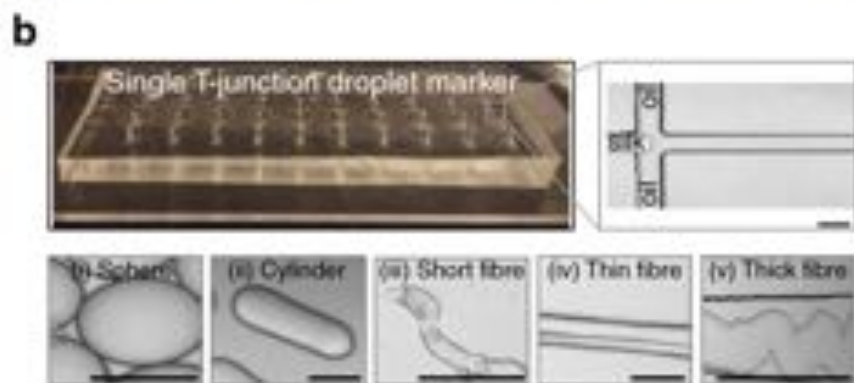
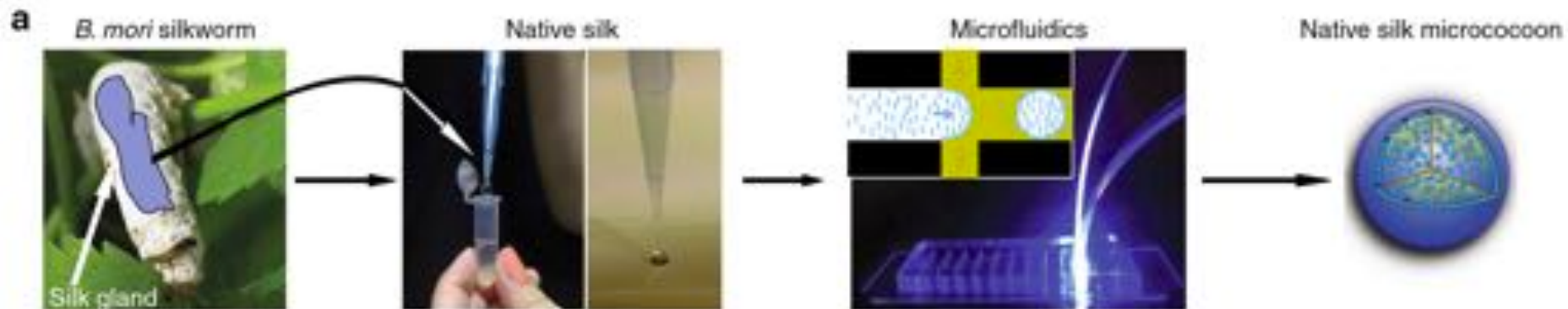


Silk Encapsulation

Microfluidic - Silk Coating

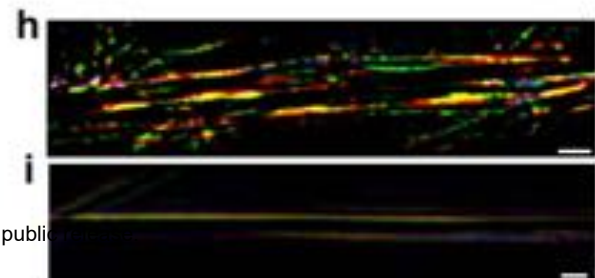
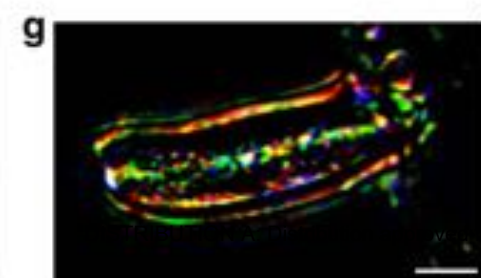
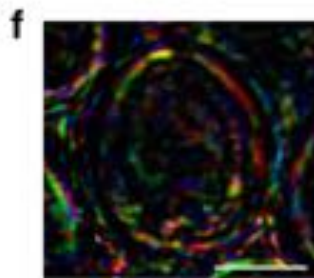
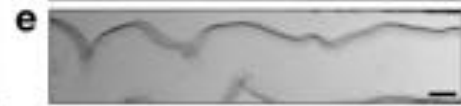
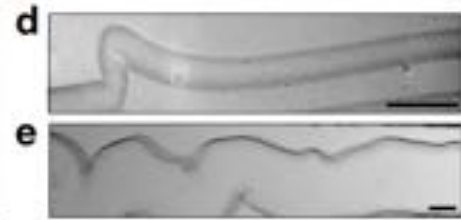
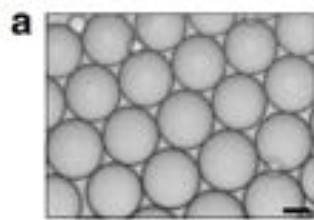
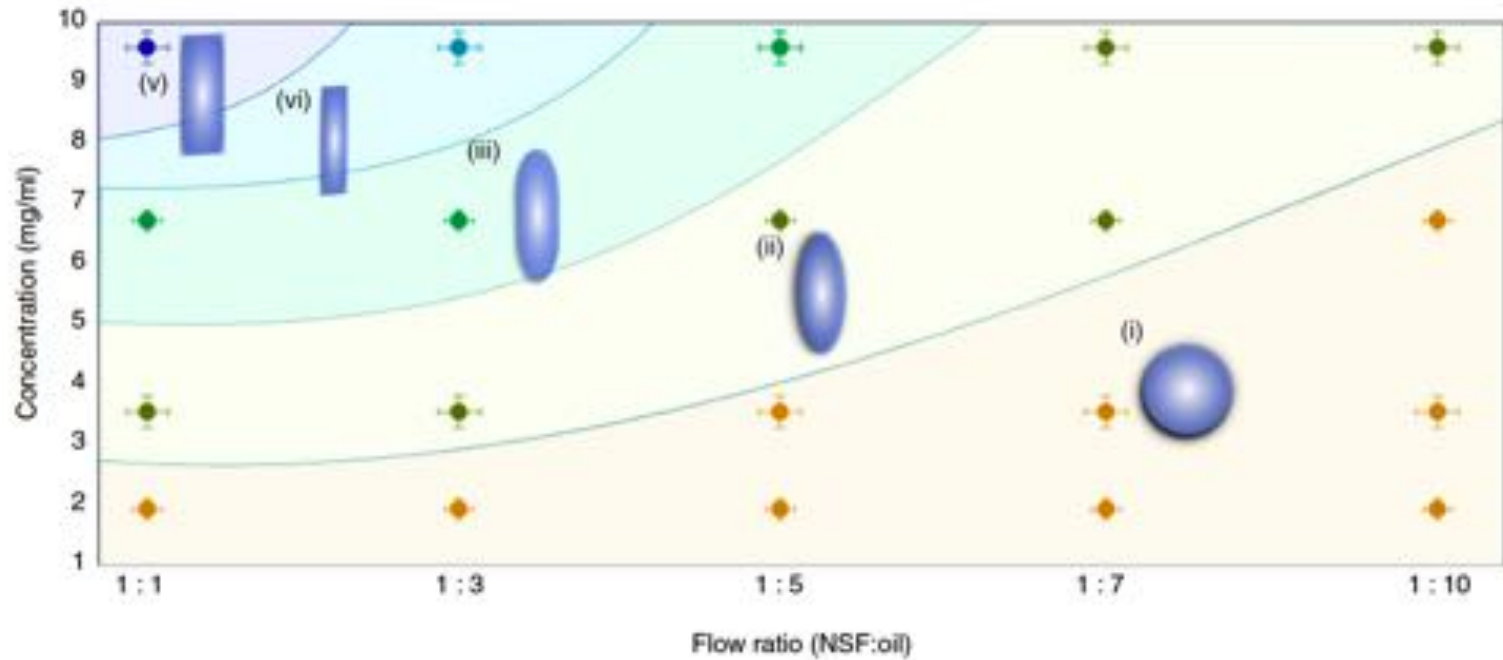
for Self-Repair & Labile Compounds/Sensors

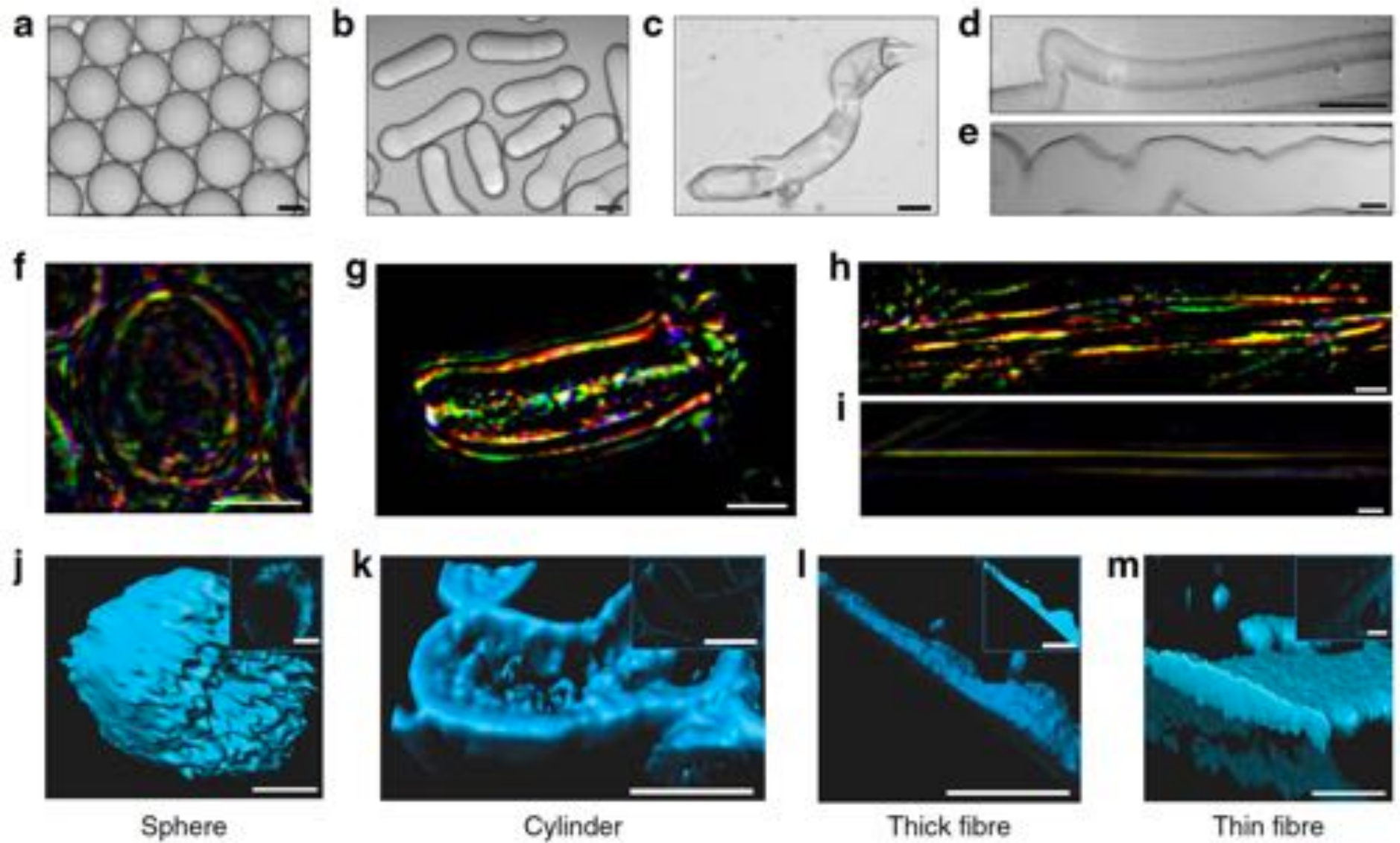




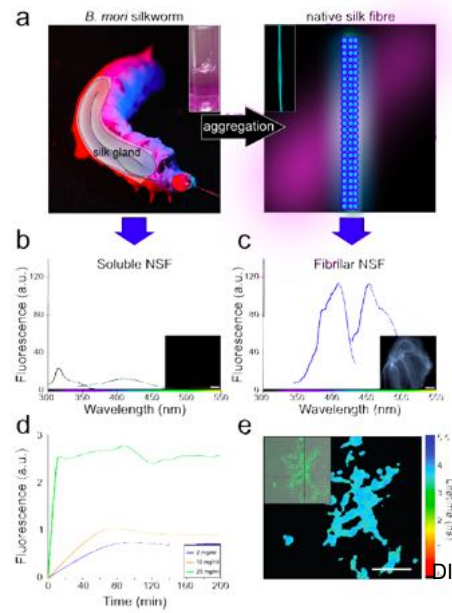
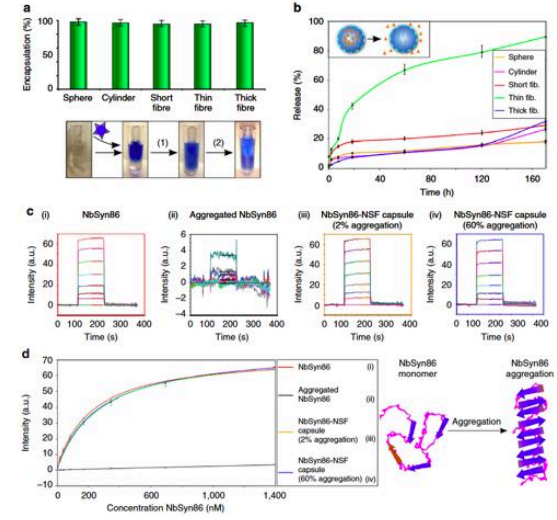
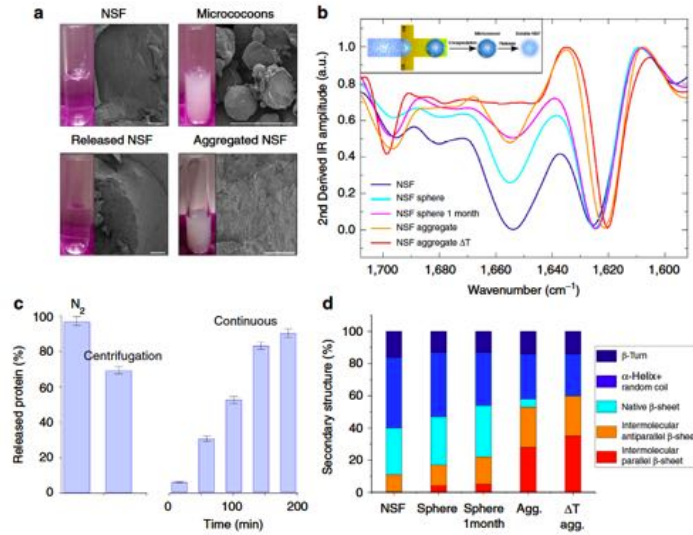
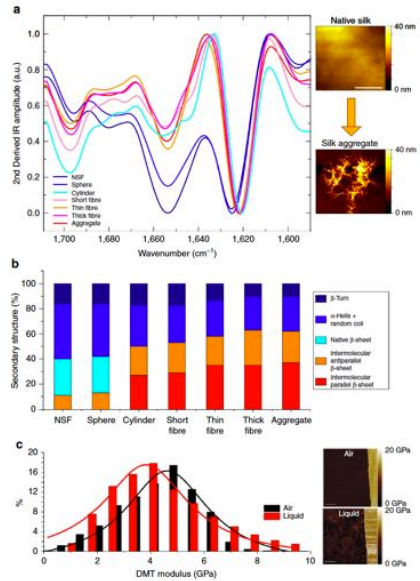
Manipulation Morphology by Flow

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Micro-Fluidic Preservation by Silk : Data



Shimanovich et al 2017 Nature Comms
DOI: 10.1038/ncomms15902

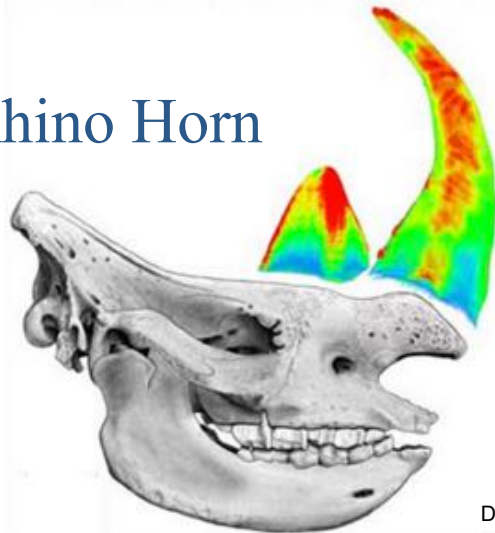
Application: Silk in Complex Composites

Micro-machines and bio-inspired Horn/Tooth

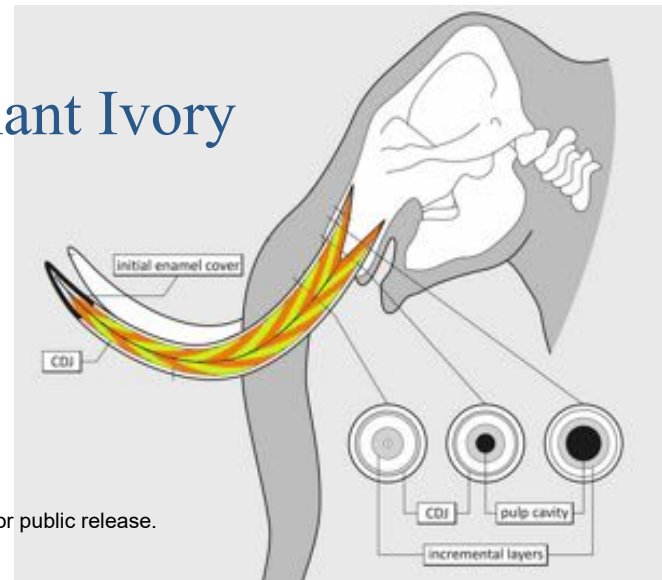
The Silk Windlass Project



Rhino Horn



Elephant Ivory

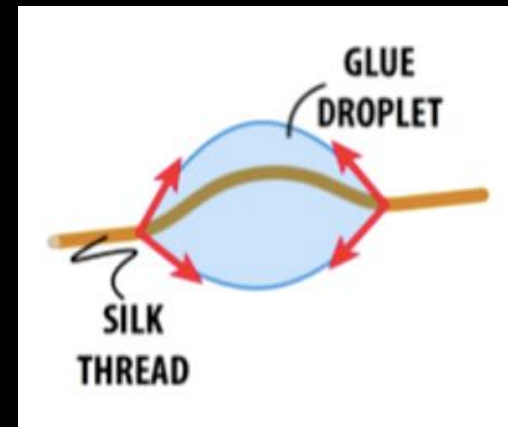
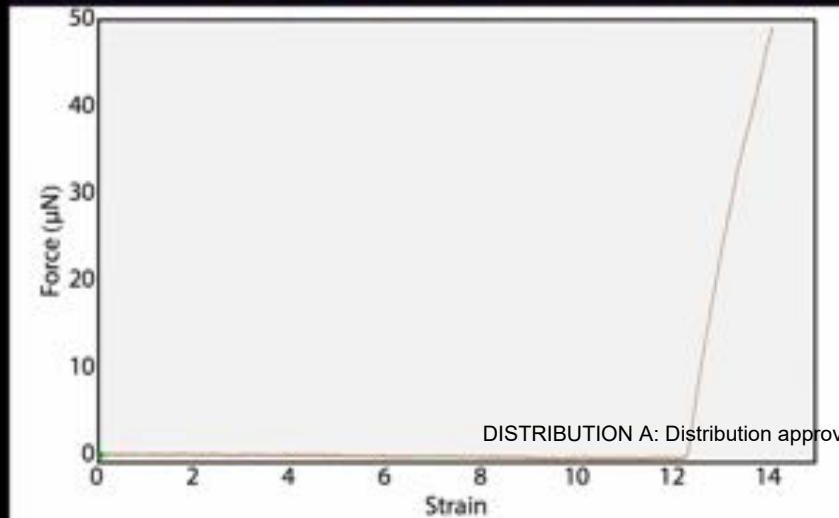
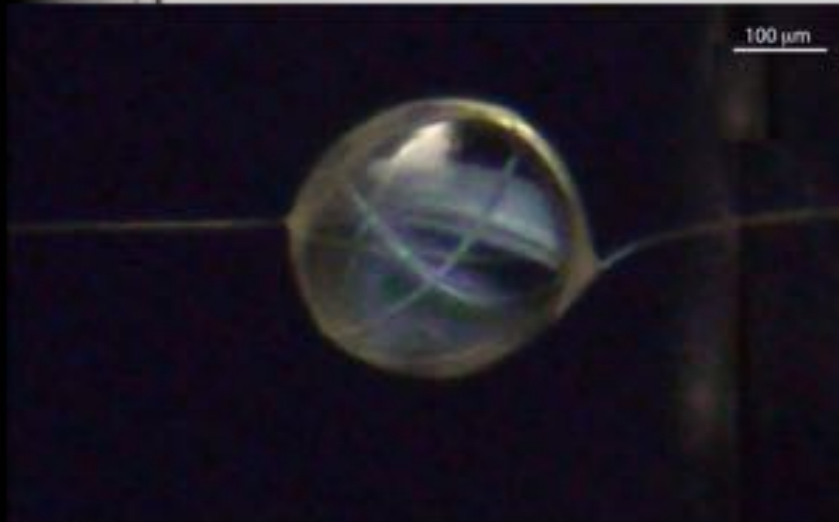


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Droplets merging and reeling in the silk



This keeps the silk thread always taut



Drop's Capillary
Compression leads to
Buckling the leads to
Coiling

Making an Artificial Winch System



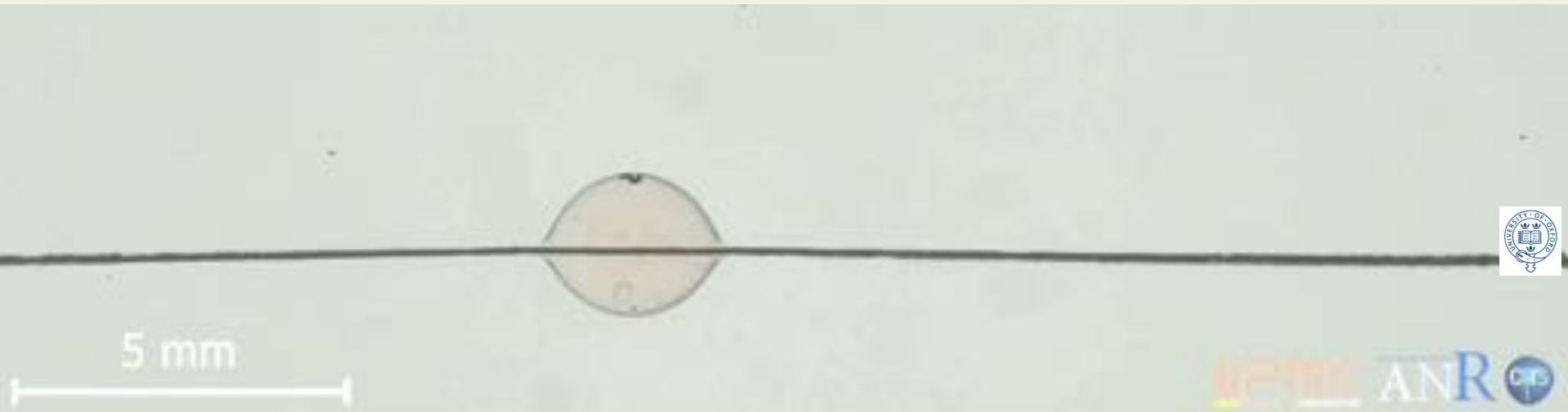
Functionalise with ??

*Shad Thaxton's
Molecules*

MURI Melatonin

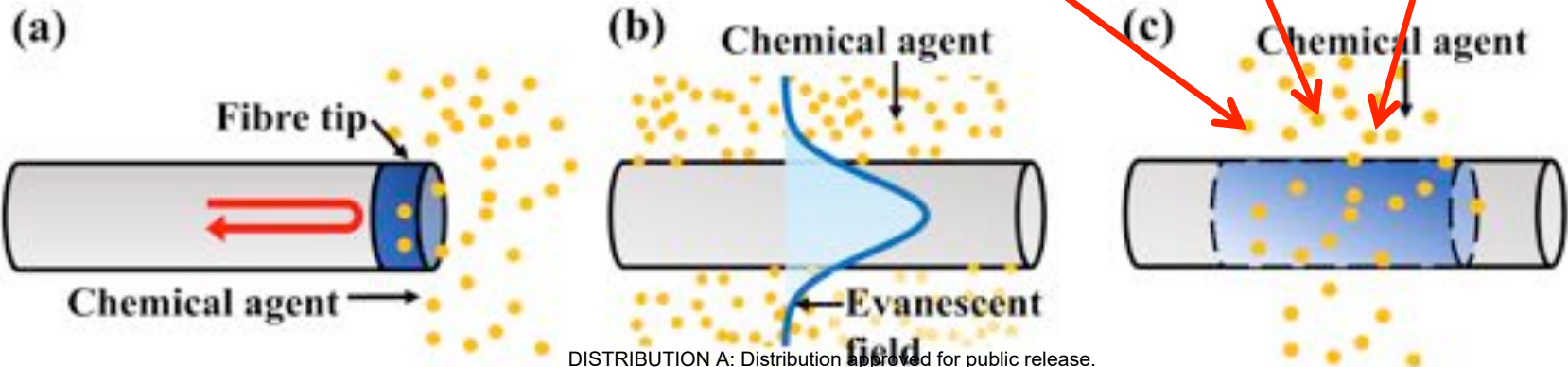
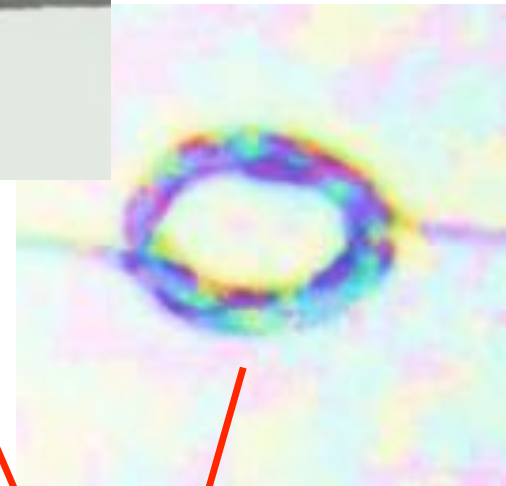
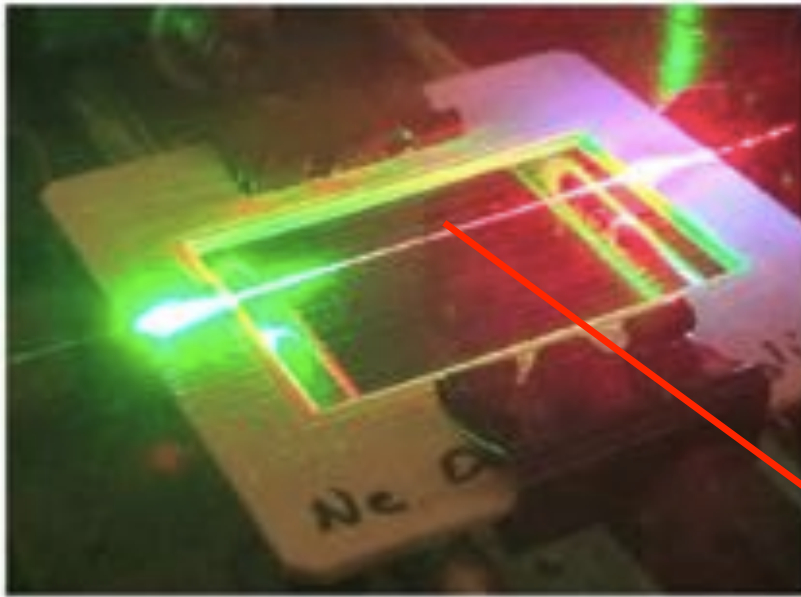
*Bruce Branchini's
Luciferins*

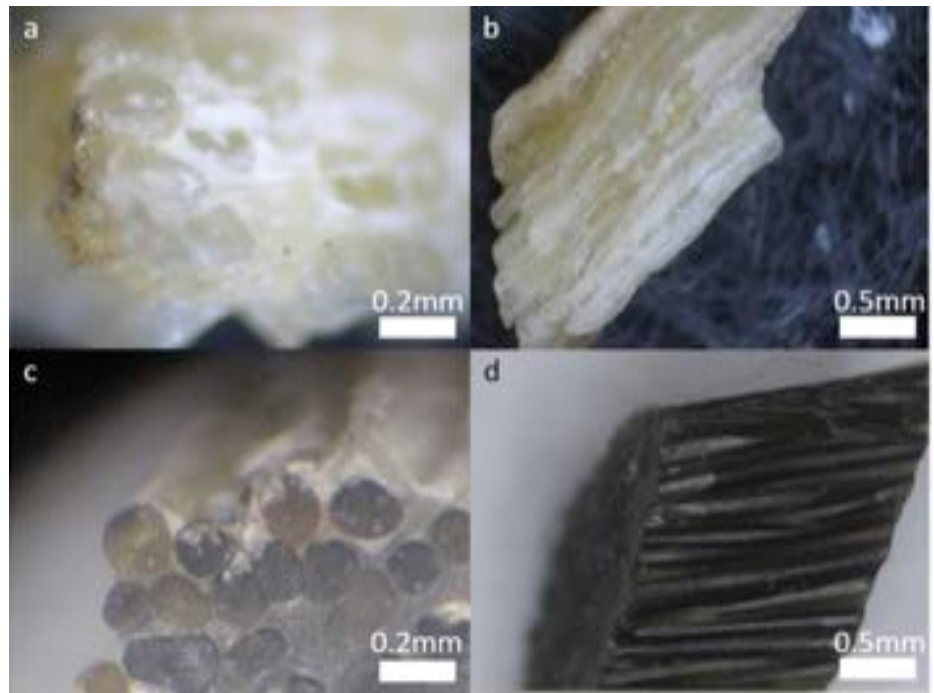
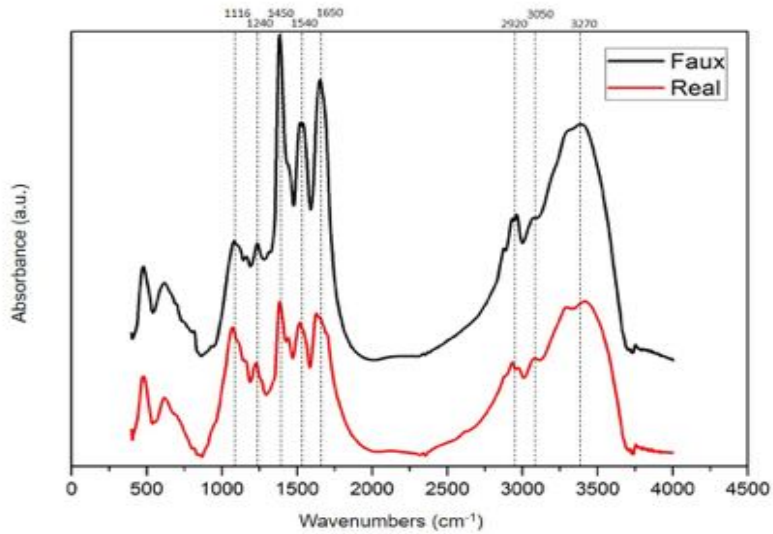
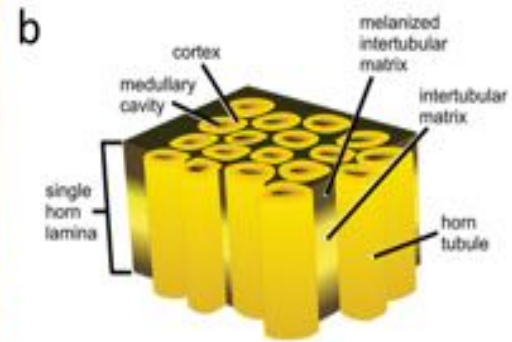
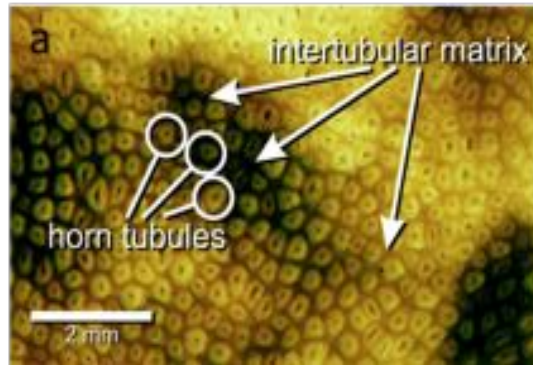
*Dimitri Deheyn's
YFP*



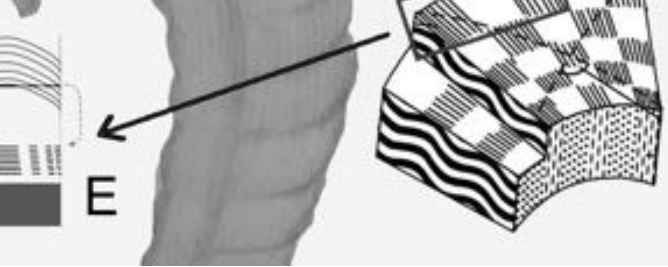
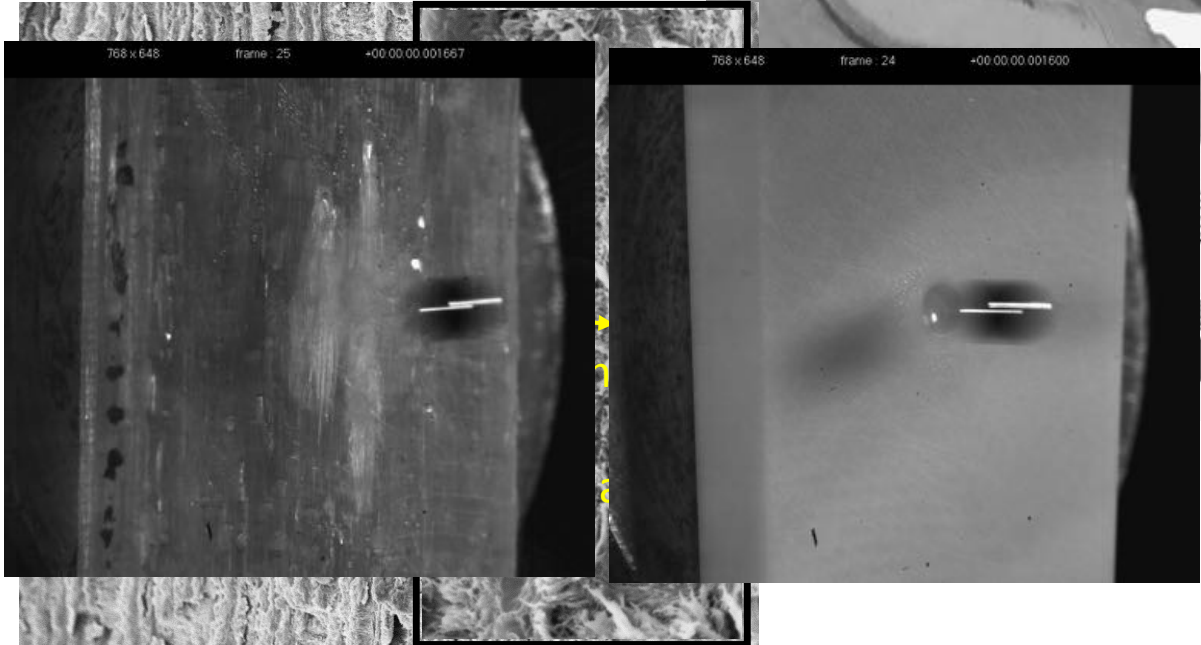
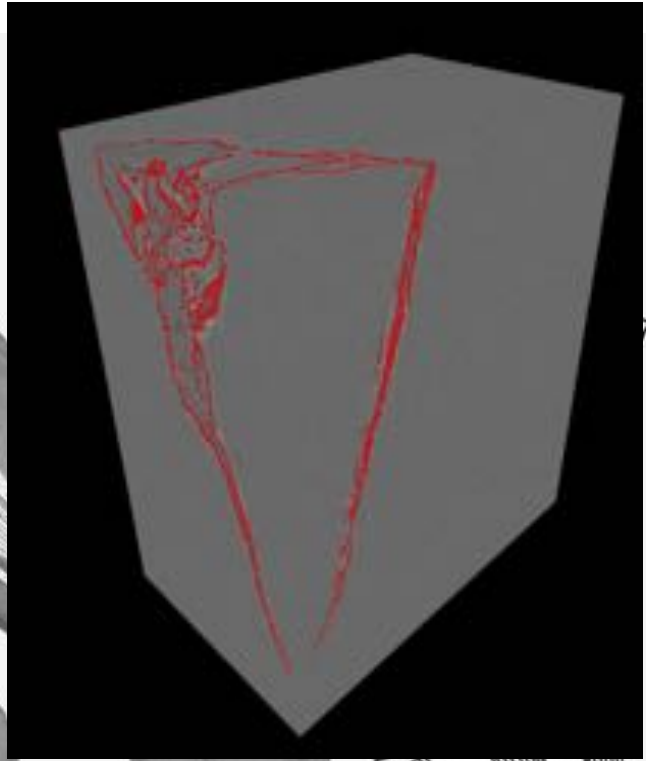
*Jeff Gilman's
Mechanophores*

Silk – Glassfibre Optic Bio-Sensors





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