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Spider silk reinforced by graphene or carbon nanotubes

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Abstract

Spider silk has promising mechanical properties, since it conjugates high strength (~1.5 GPa) and toughness (~150 J g^{-1}). Here, we report the production of silk incorporating graphene and carbon nanotubes by spider spinning, after feeding spiders with the corresponding aqueous dispersions. We observe an increment of the mechanical properties with respect to pristine silk, up to a fracture strength ~5.4 GPa and a toughness modulus ~1570 J g^{-1}. This approach could be extended to other biological systems and lead to a new class of artificially modified biological, or 'bionic', materials.

Introduction

Silkworm silks have been widely used by mankind for millennia, but only recently their mechanical properties and structure have been investigated in depth [1, 2]. An increasing number of studies also focuses on spider silk, due to its promising mechanical (~10 GPa Young's modulus, ~1.5 GPa strength [3, 4], ~100% ultimate strain [5]) and thermal properties (~400 W $m^{-1} \cdot K^{-1}$ thermal conductivity [6]), combined with biocompatibility [7] and biodegradability [5, 7, 8]. This makes it potentially useful in practical applications, such as wear-resistant lightweight clothing [9], bullet-proof vests [10], ropes [11], nets [12], bandages [13], surgical threads [14], artificial tendons or ligaments [15], biodegradable food wraps [16], or rust-free panels on vehicles [17]. E.g., in [18], researchers used individual spider silk fibres braided together to create sutures for flexor tendon repair. Enhanced silk mechanical properties could further improve the fatigue strength and lifetime of these structures.

The production of silk is key to the spiders' evolutionary success and has been perfected over 400 million years [7]. Silk is generally described as a semi-crystalline [19], biocompatible [7], composite biopolymer [5], and comprises the amino acids alanine, glycine and serine, organized into semi-amorphous helical-elastic α-chains and β-pleated nanocrystals [20, 21]. From a mechanical point of view, it is considered amongst the best spun polymer fibres in terms of tensile strength [3, 4] and ultimate strain [5], therefore toughness [11], even when compared with the best performing synthetic fibres, such as Kevlar [22]. Silk spinning involves a number of biological, chemical and physical processes [23], leading to its superior mechanical properties.

The natural presence of biominerals in the protein matrix and hard tissues of insects [24], worms [25] and snails [26] enables high strength and hardness (>500 MPa) of teeth [27, 28], jaws [28, 29], and mandibles [30, 31]. Thus, the artificial incorporation of various nanomaterials in biological protein structures to obtain improved mechanical properties should, in principle, be possible. A number of groups introduced inorganic nanoparticles [32], semiconducting crystals [33] or carbon nanotubes (CNTs) [34] on the surface of spider silk fibres, achieving an

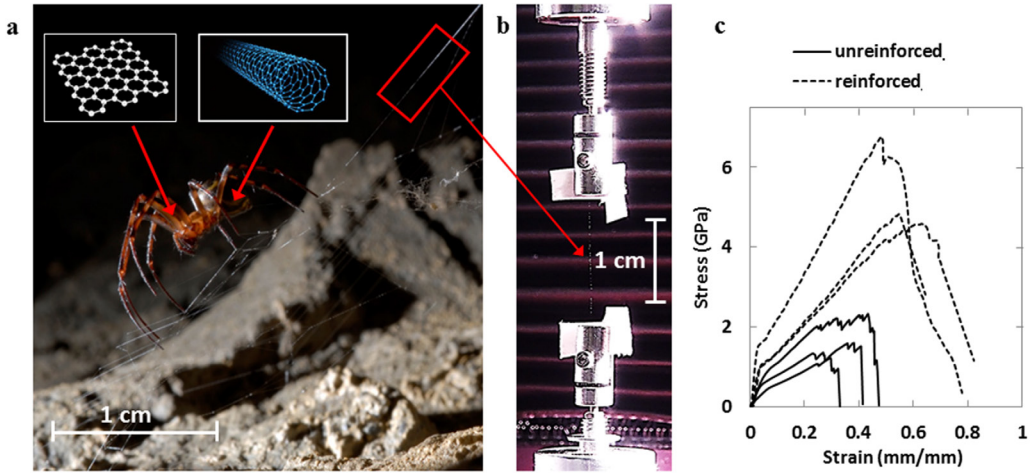

Figure 1. Schematic of the experimental procedure. (a) Spiders are fed with dispersions containing graphene or CNTs, (b) the corresponding spun silk is collected and tested in a nanotensile system; (c) stress–strain curves show improved mechanical properties when compared to pristine samples.

Table 1. Mechanical properties (average values) of RS samples.

Spider n.	Diameter (μm)	Number of threads	Young's modulus (GPa)	Ultimate strain (mm mm^{-1})	Fracture strength (MPa)	Toughness modulus (MPa)
1	0.51 ± 0.10	15	6.0 ± 3.6	0.29 ± 0.12	795.2 ± 500.1	128.2 ± 97.2
2	0.72 ± 0.15	7	27.3 ± 5.0	0.58 ± 0.23	2397.2 ± 635.5	713.0 ± 138.5
3	0.75 ± 0.11	10	13.4 ± 6.9	0.46 ± 0.40	1257.5 ± 1299.3	422.6 ± 567.6
4	0.69 ± 0.06	108	1.9 ± 0.6	1.38 ± 1.11	465.1 ± 119.0	235.6 ± 126.2
5	0.71 ± 0.06	128	3.2 ± 0.8	1.02 ± 0.30	534.7 ± 222.1	172.4 ± 77.4
6	0.72 ± 0.02	2	37.1 ± 19.6	0.28 ± 0.06	4045.9 ± 1391.6	732.1 ± 354.9
7	0.86 ± 0.07	10	15.1 ± 6.4	0.39 ± 0.07	1726.6 ± 565.3	476.4 ± 257.8
8	0.65 ± 0.05	113	2.1 ± 2.0	0.69 ± 0.40	179.7 ± 164.0	61.1 ± 79.3
9	0.51 ± 0.04	95	5.9 ± 5.0	0.53 ± 0.44	580.7 ± 482.9	205.3 ± 179.8
10	0.81 ± 0.09	48	3.0 ± 1.5	0.55 ± 0.08	281.2 ± 179.6	75.3 ± 45.8
11	0.51 ± 0.05	111	24.3 ± 13.5	0.75 ± 0.29	1969.1 ± 1158.8	764.7 ± 640.5
12	0.83 ± 0.06	17	3.1 ± 0.1	0.77 ± 0.10	173.4 ± 0.1	48.9 ± 0.1
13	0.66 ± 0.06	74	5.5 ± 5.0	0.88 ± 0.74	648.6 ± 501.9	320.3 ± 385.8
14	1.02 ± 0.03	64	3.8 ± 1.1	1.71 ± 0.87	364.0 ± 164.1	247.8 ± 111.2
15	0.54 ± 0.02	4	9.2 ± 1.5	0.26 ± 0.06	825.2 ± 182.9	101.8 ± 9.6

enhancement of toughness [35], or novel properties, such as magnetism [32] or electrical conductivity [34]. This type of reinforcement or functionalization could further make silk potentially attractive for a wide range of applications, from garment textiles [9] to sensing devices [34], from medical applications, such as suture threads [14] or tissue regeneration materials [15], to defence applications such as flak jackets [10], currently limited by the silk large deformability [36–39].

Successful attempts to improve the mechanical properties of spider silk have been limited [34, 40]. This is due to the difficulty of developing an adequate spinning methodology, balancing extrusion, drawing, yield and purity [41]. Naturally-spun fibres, obtained by forcible spinning [42], harvesting [43] or extracting spidroin (i.e. the main protein in dragline silk [2]) from glands [44], have reduced mechanical characteristics with respect to naturally-spun ones, e.g. due to the CO_2 anaesthesia of spiders [45] and the consequent loss of active control of their silk spinning [46]. From a technological point of view, wet-spinning [47], electro-spinning [48], hand-drawing [42] or microfluidic approaches [49] have been investigated to produce an artificial silk at the laboratory scale, mechanically [34], structurally [40] or chemically [49] modified with respect to the natural one. However, a critical step is still needed to reach commercial-scale.

Here, we present a method for producing reinforced spider silk directly spun by spiders after their exposure to water dispersions of CNTs or graphene, as schematically shown in figure 1. This leads to improved mechanical properties, and a toughness modulus (defined as the area under the load-displacement curve, from the origin up to fracture [50, 51], per unit mass) surpassing synthetic polymeric high-performance fibres [52] and even the current toughest 'knotted' fibres [53–55].

Table 2. Mechanical properties (average values) of the first collection of silk samples produced after exposure of the spiders to CNTs or graphene (Spiders n. 1–6 with SWNT-1, spiders n. 7–10 with SWNT-2, spiders n. 11–15 with graphene). The largest increments in the silk mechanical properties are observed for spider 5 whereas the highest absolute values are observed for spider 7.

Spider n.	Diameter (μm)	Number of threads	Reinforcement	Young's modulus (GPa)	Ultimate strain (mm mm^{-1})	Fracture strength (MPa)	Toughness modulus (MPa)
1	0.57 ± 0.04	27	SWNT-1	3.9 ± 3.9	0.62 ± 0.65	326.1 ± 150.6	66.7 ± 60.9
2	0.43 ± 0.02	4	SWNT-1	40.1 ± 48.4	0.20 ± 0.16	3914.6 ± 5038.3	587.2 ± 820.3
3	0.77 ± 0.09	72	SWNT-1	8.7 ± 6.9	0.68 ± 0.37	1195.8 ± 1037.5	387.0 ± 384.7
4	0.76 ± 0.07	44	SWNT-1	2.4 ± 0.6	0.95 ± 0.52	579.4 ± 313.1	187.6 ± 66.7
5	0.78 ± 0.08	4	SWNT-1	37.9 ± 4.4	0.60 ± 0.28	3907.2 ± 874.1	1144.0 ± 555.3
6	0.84 ± 0.068	12	SWNT-1	9.6 ± 5.2	0.50 ± 0.24	954.3 ± 278.2	210.2 ± 87.3
7	1.00 ± 0.12	62	SWNT-2	47.8 ± 18.0	0.75 ± 0.09	5393.5 ± 1202.4	2143.6 ± 684.6
8	0.42 ± 0.08	34	SWNT-2	3.1 ± 1.1	0.41 ± 0.11	315.7 ± 124.7	47.8 ± 22.8
9	0.42 ± 0.04	82	SWNT-2	19.3 ± 6.3	0.34 ± 0.14	2034.9 ± 212.8	419.8 ± 96.2
10	0.81 ± 0.09	48	SWNT-2	0.2 ± 0.0	0.32 ± 0.02	20.1 ± 3.5	2.6 ± 1.8
11	0.67 ± 0.02	43	GS	0.8 ± 0.6	0.33 ± 0.19	58.0 ± 22.9	7.9 ± 3.4
12	0.74 ± 0.13	41	GS	4.9 ± 1.1	0.43 ± 0.06	607.5 ± 219.2	148.5 ± 71.6
13	0.66 ± 0.06	74	GS	3.1 ± 1.7	0.52 ± 0.37	421.8 ± 251.3	130.3 ± 129.0
14	1.02 ± 0.03	64	GS	0.4 ± 0.2	0.29 ± 0.08	45.9 ± 17.1	6.0 ± 2.8
15	0.61 ± 0.06	14	GS	13.0 ± 6.5	0.43 ± 0.24	1245.6 ± 559.4	254.7 ± 164.3

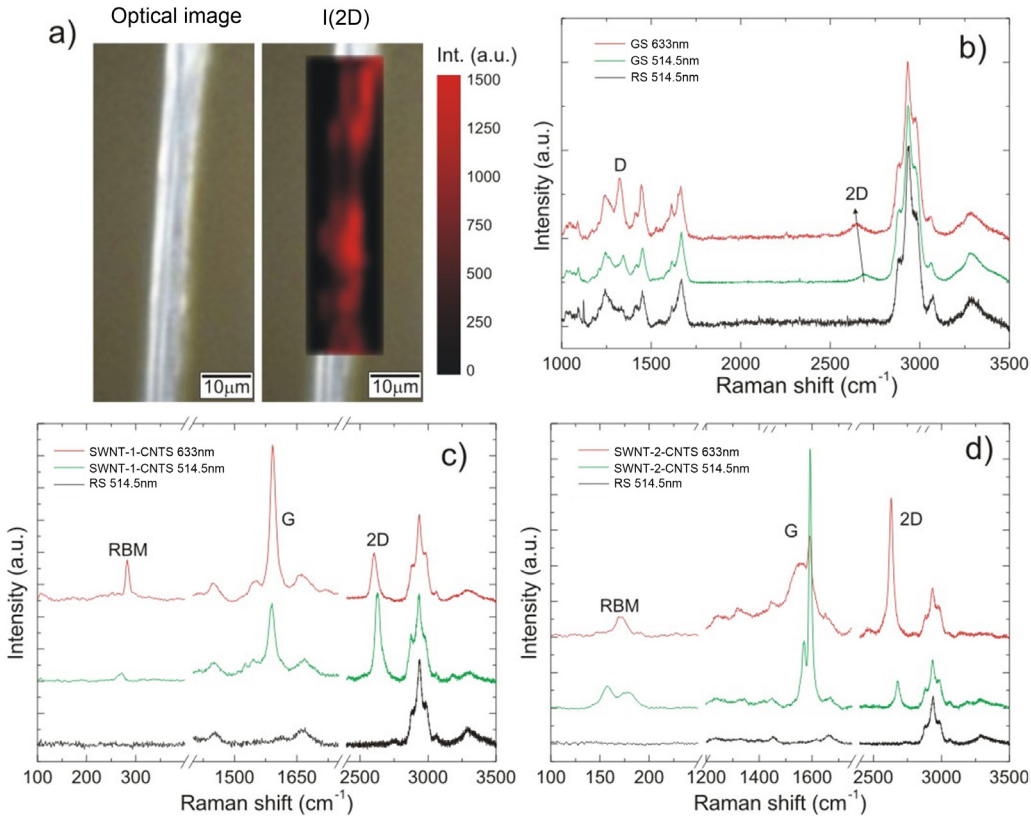

Figure 2. (a) Optical picture of a spider silk fibre suspended by a mechanical support (left) and map of $I(2D)$ overlapped to the optical picture of the spider silk spun by spiders after exposure to graphene (right). (b) Raman spectra of RS (black line, at 514.5 nm), GS at 514.5 nm (green line) and 633 nm (red line). Raman spectra of (c) SWNT-1-CNTS (d) SWNT-2-CNTS at 514.5 nm (green line) and 633 nm (red line) excitation wavelengths.

Results

Two types of CNTs are used in this study. The first is CoMoCAT [56] (~0.6–1.35 nm diameter) single-wall nanotubes (SWNT-1). The second is electric arc discharge SWNTs (P2) from Carbon solutions inc. (SWNT-2). CNT dispersions are prepared by adding 1 mg/10 ml weight-to-volume ratio of each CNT source to an aqueous solution of 2% w/v sodium deoxycholate (SDC, from Sigma-Aldrich Ltd) in deionised water. This surfactant is not harmful for the spiders, as discussed in the supplementary information (SI) (stacks.iop.org/TDM/4/031013/mmedia). De-bundling is obtained via ultrasonication using a Branson Ultrasonic Processor

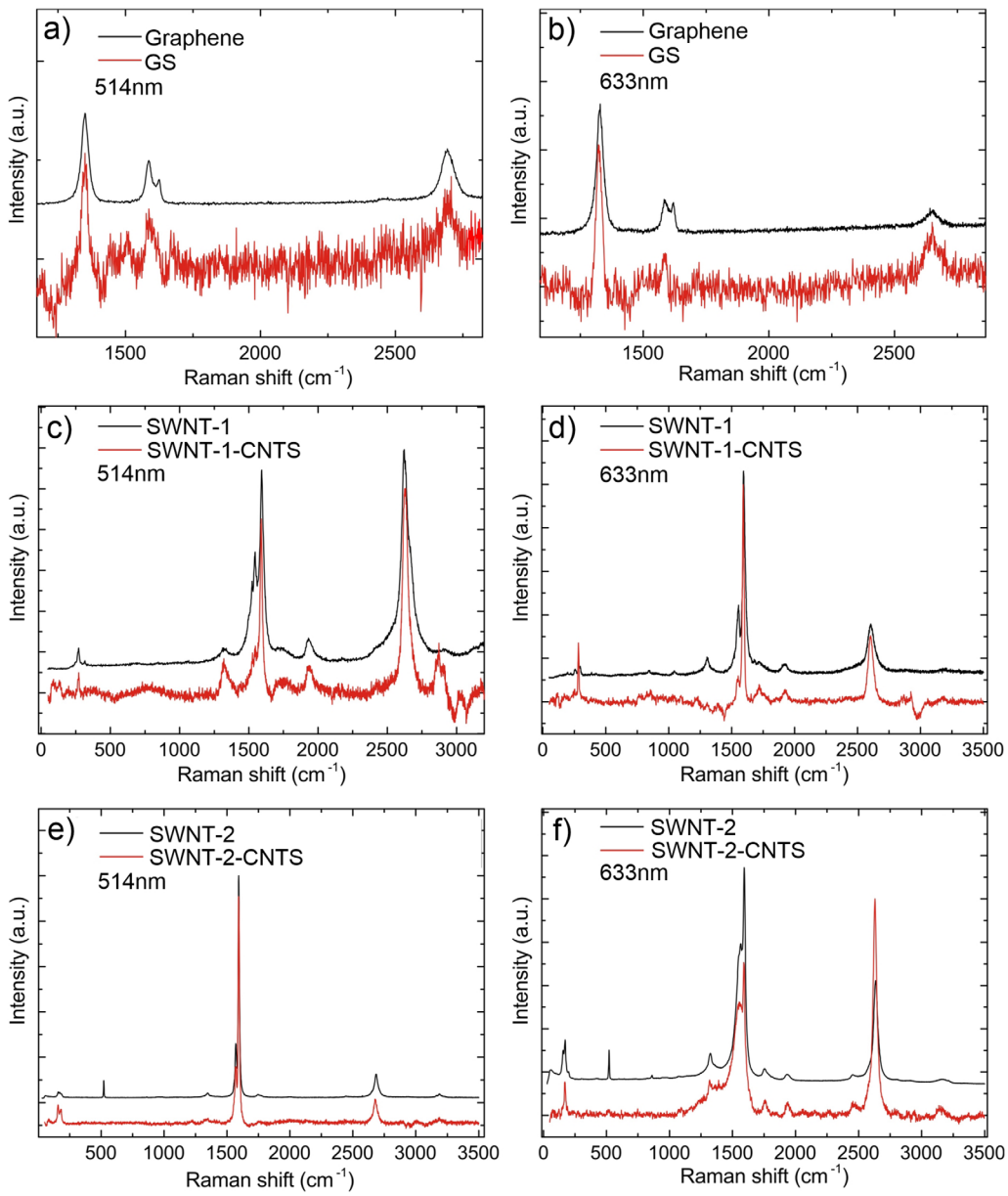

Figure 3. Raman spectra of graphene (black line) and GS (red line) at (a) 514.5 nm and (b) 633 nm. Raman spectra of SWNT-1, SWNT-2 and SWNT-1-CNTS and SWNT-2-CNTS at (c) and (e) 514.5 nm and (d) and (f) 633 nm excitation wavelengths.

for 2 h (450 kW at 20 kHz). The dispersions are ultracentrifuged using a TH-641 swinging bucket rotor in a Sorvall WX-100 at 200 000 g for 2 h at 18 °C to remove bundles and other impurities, such as amorphous carbon and catalyst residuals [57]. The supernatant of the two dispersions after ultracentrifugation is collected using pipettes and used for the characterization. Graphite flakes are sourced from Sigma Aldrich Ltd. 100 mg are dispersed in 10 ml water with 2% volume to weight (v/w) SDC. The dispersion is then ultrasonicated for 10 h and subsequently ultracentrifuged, exploiting sedimentation-based separation (SBS) [58] using a TH-641 swinging bucket rotor in a Sorvall WX-100 ultracentrifuge at 5000 rpm for 30 min. After ultracentrifugation, the supernatant is extracted by pipetting. The concentration of graphitic flakes is determined from the optical absorption coefficient at 660 nm [59]. A full optical and spectroscopic characterization of the samples is presented in SI. This shows that the samples consist of ~60% single- (SLG) and ~40% few-layer (FLG) graphene flakes [60].

21 spiders of three species were selected (*Pholcidae Holocnemus*, *Pholcidae Pholcus* and *Theridiidae Steatoda*) as described in the SI. The spiders are exposed to the aqueous dispersions by spraying them in a corner of the box they are contained in, avoiding intentional direct spraying on the animals. The dragline silk is collected from 2 to 12 days later, in order to allow sufficient time for ingestion of the aqueous dispersions and the production of silk. 29% of the spiders died before the first silk collection, and a further 24% after 12 days, during which starvation could have come into play. The silk fibres consist of multiple threads of approximately circular cross-section. The average diameter of the single threads is determined for each sample through field emission

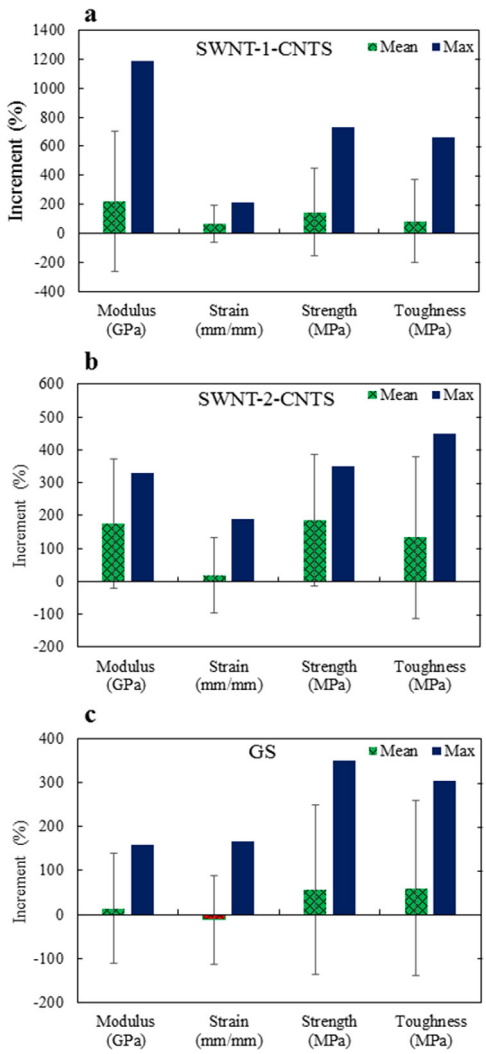

Figure 4. Percentage increment (green bars) or decrement (red bars) of the mechanical properties measured from RS and (a) SWNT-1-CNTS, (b) SWNT-2-CNTS or (c) GS. To calculate toughness values, a mean silk density value of 1.34 g cm^{-3} is used.

scanning electron microscopy (see SI), at two different cross-sections along their length. The number of threads in each fibre is also counted. The presence of CNTs and graphene is monitored by Raman spectroscopy.

Nano-tensile tests are performed under controlled conditions as described in the SI. Samples are prepared by fixing silk fibre ends to 'C' shaped cardboard holders (then cut after mounting in the sample holder, figure 1(b)), and subjected to traction up to failure in an Agilent T150 nanotensile system at a constant strain rate of 0.1% s^{-1}, consistently with previous studies on spider silk mechanics [4, 61–63]. The stress σ, strain ε, and Young's modulus E, are calculated as $\sigma = F/A_0$, $\varepsilon = \Delta l/l_0$, $E = \mathrm{d}\sigma/\mathrm{d}\varepsilon|_0$, where F is the force measured by the nanotensile system (see SI for details), A_0 is the cross-section area of the fibre, l_0 its initial length, and Δl the change in fibre length during the test. The area underlying the stress–strain curve corresponds to the energy per unit volume required to break the fibre, the so-called toughness modulus T, also alternatively given in energy per unit mass, as derived by dividing the energy per unit volume by the density of the material: $T = \frac{1}{\rho}\int \sigma \mathrm{d}\varepsilon$ [53].

The diameter of the cross-sectional area of the silk fibres is between ~5 and ~10 μm, as obtained by multiplying the mean value of the measured cross-sectional area of the threads by their total number. The resulting diameters and number of threads for each fibre are reported in tables 1 and 2 for the reference spider silk (RS) and that collected from spiders exposed to dispersions of graphene (GS) and SWNTs (CNTS).

Figure 2 shows an optical image of a suspended fibre (figure 2(a)) and compares the Raman spectra of RS with that of GS (figure 2(b)) and CNTS (figures 2(c) and (d)). The RS Raman spectrum comprises several peaks in the 1000–1800 cm^{-1} and 2700–3500 cm^{-1} regions. The peaks at ~1088 and 1160 cm^{-1} are characteristic of the n(C–C) skeletal band of polypeptide chains [64, 65]. Two intense bands are also seen at ~1230 cm^{-1}, characteristic of amide III groups in π-sheets structured proteins [66, 67] and at ~1444 cm^{-1} assigned to CH_2 bending modes, both bands typically found in the Raman spectrum of spider silk [68]. The peaks at 1615 cm^{-1} and 1665 cm^{-1} are assigned to n(CO) amide I bands characteristic of the π-sheets configuration for the polypeptide backbone [66, 68]. The Raman peaks in the region 2700–3500 cm^{-1} are typical of C–H and N–H vibrations [67].

Raman spectra from GS and CNTS samples are shown in figure 3. The spectra are normalized with respect to the C–H band at ~2934 cm^{-1}, the most intense in RS. The normalized RS spectrum is then subtracted from the GS and CNTS ones. Figures 3(a) and (b) show that the spectrum of the flakes in the dispersion is compatible with that of GS. At both 514 and 633 nm, the D to G and 2D to G intensity ratios, I(D)/I(G) and I(2D)/I(G), as well as the positions of the G and 2D peaks, Pos(G) and Pos(2D), are very similar. The comparison indicates that graphene detected in GS has a similar level of disorder [69–72] as the original material. The same holds for CNTS. Figures 2(c)–(f) show that the spectra of the original SWNTs and those measured on CNTS are similar, indicating a negligible change in the structural properties.

A summary of the mechanical properties of RS, GS and CNTS is reported in tables 1 and 2, and typical stress–strain curves of the silk fibres are presented in figure 1(c) and in the SI. Scatter in the data is large, mainly due to the variability in the properties of the collected silk samples, deriving from spiders of different species and ages (see SI), but also from factors such as the sensitivity of silk density to humidity [73]. However, fibre slippage in the loading frame can be excluded, since this would be noticeable in the stress–strain curves, given the high-sensitivity (50 nN load resolution and 0.1 nm displacement resolution) of

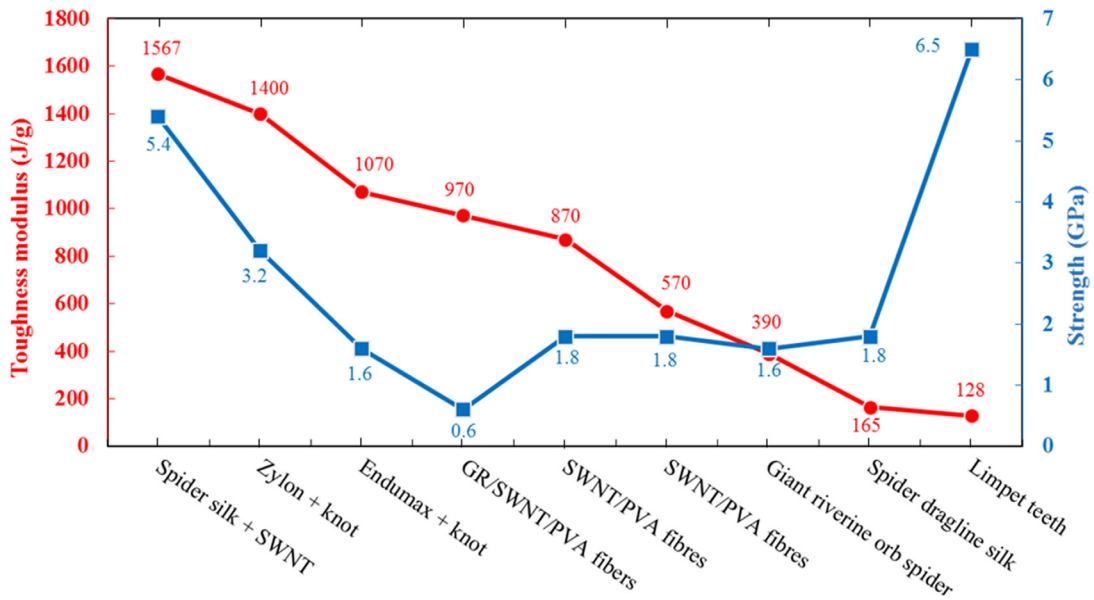

Figure 5. Toughness modulus and strength of different materials and composites.

the nanotensile testing system. Ultimate strain values have smaller scatter, since they are not dependent on parameters such as fibre cross-sectional area. In CNTS and GS, the scatter is greater due to the varying CNTs and flakes concentrations, since there is no control on the nanoparticle uptake mechanism at this stage. Apart from this, there is an intrinsic variability of mechanical properties of different samples of the same silk, in line with previous studies [62, 74–76].

To avoid these problems, we focus on the variation in mechanical properties of the silk fibres from individual spider specimens before and after exposure. The average and maximum variations are shown in figure 4. Fracture strength, Young's modulus and toughness increase on average between 80 and 220% in CNTS and between 15% and 60% for GS. The highest fracture strength and Young's modulus increments are +731% (3.9 GPa) and +1183% (37.9 GPa) for CNTS, with an increment ~+663% in toughness (2.1 GPa). This corresponds to 1567 J g^{-1}, calculated using an average silk density value ~1.34 g cm^{-3} [53], neglecting changes due to humidity or the presence of CNTs or graphene, as discussed in the SI, with a 41% decrement of ultimate strain (0.6 mm mm^{-1}). This should be compared to the toughest silk fibres found to date, having a toughness ~520 MJ m^{-3} [62], and a strength ~1.65 GPa [62]. The combination of increment in toughness and decrement in ultimate strain is peculiar and fundamental in applications such as parachutes or bullet-proof vests, where high performance textiles are required to stop bullets in millimetres [77]. The second highest increments ~+350% (2.0 GPa) in fracture strength and +330% (19.3 GPa) in Young's modulus are found for SWNT-2-CNTS, corresponding to an increment ~+204% in toughness (0.4 GPa), with a decrement ~36% in ultimate strain (0.3 mm mm^{-1}). A smaller, but still significant, increment is also observed for some of the fibres containing GS, with +151% (1.2 GPa) in fracture strength and +142% (13.0 GPa) in Young's modulus, corresponding to an increment of both toughness and ultimate strain of +250% (0.3 GPa) and +166% (0.4 mm mm^{-1}), respectively.

We note that Wang *et al* [78] reported a similar approach to that described here, based on the initial posting of the present manuscript [79], cited as reference [19] in [78] and applied it to silkworm silk. This demonstrates that our approach is reproducible in other biological systems. However, Wang *et al* [78] achieved far inferior mechanical properties to those reported here. Indeed, in the best case we get here a strength ~5.4 GPa and toughness modulus ~1567 J g^{-1}, whilst Wang *et al* [78] achieves maximum values ~0.6 GPa and ~400 J g^{-1}, respectively. This is mainly due to lower mechanical properties of silkworm silk with respect to spider silk [1–4].

Discussion

Figure 5 compares the strength and toughness of various natural and artificial materials. The best fibre obtained in this study displays higher strength than high-performance polymeric fibres like Zylon or Endumax [52, 53], or SWNT/carbon-reinforced polymeric fibres [80–82], and is surpassed only by T1000® Carbon fibres [83] or goethite nanofibers found in limpet teeth [27]. At the same time, its toughness is significantly higher than the toughest spider silks found in nature [62, 75] or high-toughness SWNT/PVA fibres [80]. Indeed, it exceeds the previous largest recorded toughness value for synthetic polymer fibres or CNT microfibres with integrated knots as energy dissipators [54].

Molecular Dynamics simulations reported in the SI show that CNTs have the strongest interaction with the mannose-associated serine protease 1 (MASP1) spider silk fibers, in the amorphous region where

they affect the structure of the bundle. The measured strength and stiffness data can be compared to analytical predictions for the reinforcing effect of graphene or CNTs on silk using the direct and inverse rule of mixtures [84] and simulations using the Hierarchical Fibre Bundle Model [85] (see SI). These can estimate the concentration of reinforcements by fitting the corresponding experimental strength values, giving equivalent volume fractions in GS and CNTS between 1% (spider 15) and 15% (spider 7), with an average ~7%.

Graphene flakes appear to be a less effective reinforcement than CNTs (figure 4(c)). This could be due to the shape of the flakes. The lateral characteristic dimensions of our flakes (~200–300 nm), two orders of magnitude larger than the characteristic CNT diameter, could give rise to less efficient load transfer, since their longitudinal dimension could be smaller than the critical length (of the order of microns and dependent on the interface properties) predicted by the shear lag theory for optimal load transfer [86]. Inefficient load transfer could also be due to the crumpled configuration of the flakes or a larger misalignment with respect to CNTs. Finding solutions for these problems could potentially enable GS with mechanical properties superior to CNTS, thanks to the two surfaces available for load transfer in flakes [86]. Tuning the constitutive law of the silk could also maximize the robustness of an entire structure [87].

Conclusions

Spiders placed in an environment with water solutions containing nanotubes or graphene may produce dragline silk with enhanced mechanical properties, realizing the highest fibre toughness to date, combined with a strength comparable to that of the strongest carbon fibres or of limpet teeth. Our proof-of-concept experiment paves the way to exploiting the naturally efficient spider spinning process to produce reinforced silk fibres, thus further improving one of the most promising silk materials, as compared to synthetic recombinant silks. This procedure of natural integration of reinforcements in biological structural materials could also be applied to other animals and plants, leading to a new class of 'bionicomposites' for innovative applications.

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In the Discussion on page 6 the definition of MASP 1 was 'mannose-associated serine protease 1 (MASP 1)'. It should be 'major ampullate silk protein 1 (MASP 1)'.

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Spider silk reinforced by graphene or carbon nanotubes - Supplementary Information

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S1. Collection of spiders

We randomly collected 21 Pholcidae spiders (male and female of different ages). The sampling site was Torrente Chisone, between Macello and Garzigliana, Province of Torino, Italy (Geographical coordinates: 44.844 North, 7.385 East). Each spider was gently and individually segregated in a box of 19 x 12.5 x 7.5 cm^3 with four air inlets, a transparent top, white bottom and lateral sides. Spiders were transferred within 3 days from capture under an extractor fan with controlled ambient condition temperature and humidity (21.0 ± 0.2 °C and 54.3 ± 0.7 %) where they were kept during the entire experimental procedure. The procedure employed in the present study is in accordance with the Italian regulations on animal protection [1], with the relevant EU legislation and guidelines on the ethical use of animals and is approved by the local Authority Veterinary Service.

After 5 days RS samples were collected from 15 spiders. Subsequently, spiders were exposed to aqueous dispersions of graphene and CNTs, as schematically shown in Fig S1 and detailed in Table S1. We used sodium deoxycholate as surfactant for both exfoliation and dispersion of graphite and for the de-bundling of CNTs. Sodium deoxycholate is a natural bile salt [2]. Its acid, i.e., the deoxycholic acid, is one of the secondary bile acids produced in the intestine from the salts of glycocholic and taurocholic acid by means of bacterial enzymes [3]. Less than half of the sodium deoxycholate is reabsorbed by the intestine and is returned to the liver where it is conjugated and released into the gall bladder [4]. Sodium deoxycholate is often used as a biological detergent to solubilize cellular and membrane components and lyse cells [2]. Sodium deoxycholate mixed with phosphatidylcholine is used in mesotherapy injections to produce lipolysis, and has been used as an alternative to surgical excision in the treatment of lipomas [5]. Thus, we assume, due to the nature and the biological use of the surfactant, that sodium deoxycholate is not harmful for the spiders.
Nine spiders (1, 4, 5, 7, 9, 11, 13, 14, 15) started to spin silk after 2 days, while six took 12 days (2, 3, 6, 8, 10, 12). The dragline silk was then collected. Spiders 1-6 were treated with SWNT-1, 7-10 with SWNT-2, and 11-15 with graphene, as shown in Fig. S1c and Fig. S1d. After 42 days, dragline silk was collected for the second time. The silk is in the form of a single fiber composed of multiple threads. Multiple samples are obtained by cutting the fiber into 20mm-length strands for tensile tests and for the measurement of the cross-sectional area, following the procedure described in [6]. This

suggested to take adjacent samples to ensure reproducibility of fiber properties and to use one out of five samples (instead of three as here) as control to measure the fiber cross-sectional area by Field Emission Scanning Electron Microscopy (FESEM). In our work, the cross-sectional area of the fiber is determined using a FESEM without sputter coating.

ID	**Family**	**Genus**	**Species**	**sex**	**Material**
1	Pholcidae	Holocnemus	pluchei	M	SWNT-1
2	Pholcidae	Holocnemus	pluchei	M	SWNT-1
3	Pholcidae	Pholcus	opilionoides	M	SWNT-1
4	Pholcidae	Pholcus	phalangioides	M	SWNT-1
5	Theridiidae	Steatoda	sp.	M	SWNT-1
6	Theridiidae	Steatoda	triangulosa	F	SWNT-1
7	Pholcidae	Pholcus	opilionoides	M	SWNT-2
8	Pholcidae	Holocnemus	pluchei	M	SWNT-2
9	Theridiidae	Steatoda	sp.	M	SWNT-2
10	Pholcidae	Holocnemus	pluchei	M	SWNT-2
11	Pholcidae	Holocnemus	pluchei	M	GRAPHENE
12	Pholcidae	Holocnemus	pluchei	M	GRAPHENE
13	Pholcidae	Holocnemus	pluchei	M	GRAPHENE
14	Pholcidae	Holocnemus	pluchei	M	GRAPHENE
15	Pholcidae	Holocnemus	pluchei	M	GRAPHENE

Table S1: *Spider types used in experiments and nanomaterial solution they were exposed to.*

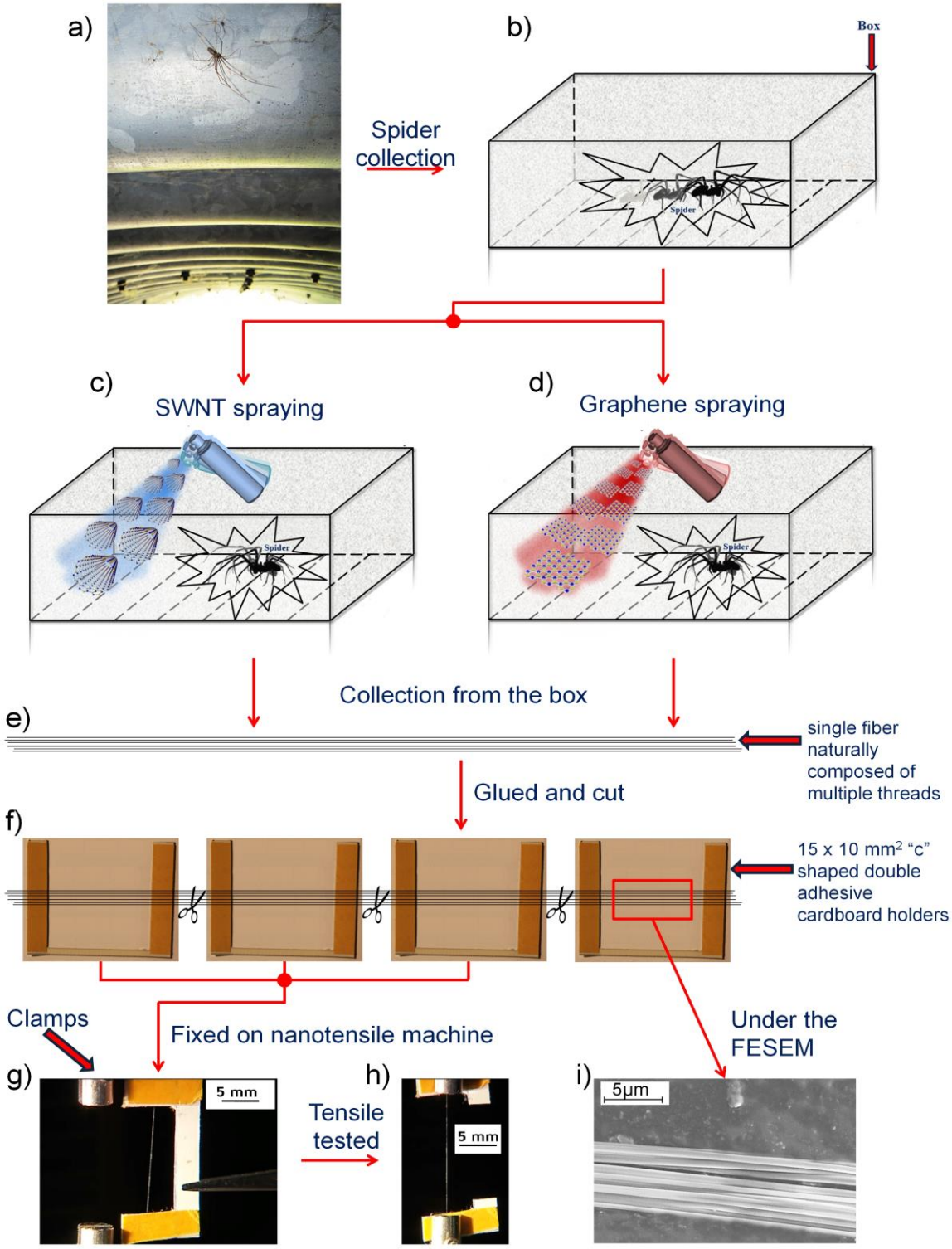

Figure S1: ***Schematic illustration of the experimental procedure.*** *(**a**) Sampling site and experimental box for the collection of (**b**) RS samples and, after spraying with (**c**) CNTs or (**d**) graphene dispersions. (**e**) Collection of silk in the form of a single fibre, composed of multiple threads, from which multiple samples are obtained by fixing the ends of the fibre to 15 x 10 mm^2 "c" shaped double adhesive cardboard holders and (**f**) cutting the fibre into four shorter pieces, (**g**) mounted on nanotensile machine clamps (**h**) for nanotensile tests and (**i**) for measuring the fibre cross-sectional surface area with a FESEM. Scale bar 5 μm.*

S3. Characterization

S3.1 Optical absorption Spectroscopy

S3.1.1 Optical absorption spectroscopy of SWNTs

Optical absorption spectroscopy (OAS) reveals various properties of SWNT dispersions such as transition energies [7, 8] bundling [9] and concentration [10].

Spectra are acquired in a Perkin Elmer 950 with 1nm resolution. Measurements are carried out in the range 400-1300nm, limited by the strong absorption features of water [11]. However, this range is sufficient to cover the first and second excitonic transitions of s-SWNTs [12, 13] and the first transition of m-SWNTs, for CoMoCAT [12, 13] and the second and third transitions of s-SWNTs and first of m-SWNTs [14, 15] for the P2 samples. Absorption from solvent and surfactants is subtracted, by measuring solutions with only solvent and surfactant.

The assignment of the optical transitions is based on the empirical Kataura plot of Ref. [15]. This gives values of optical transition frequencies versus chirality for SWNTs in aqueous surfactant dispersions, and is more appropriate than Kataura plots theoretically derived from tight binding and other models [16].

The OAS of SWNT-1 and SWNT-2 are reported in Fig. S2. SWNT-1 have sharper peaks with respect to SWNT-2 sample. The OAS of SWNT-1 shows the M_{11} region as well as the eh_{11} and eh_{11} regions, while SWNT-2 shows the M_{11} region as well as the eh_{22} and eh_{33} regions.

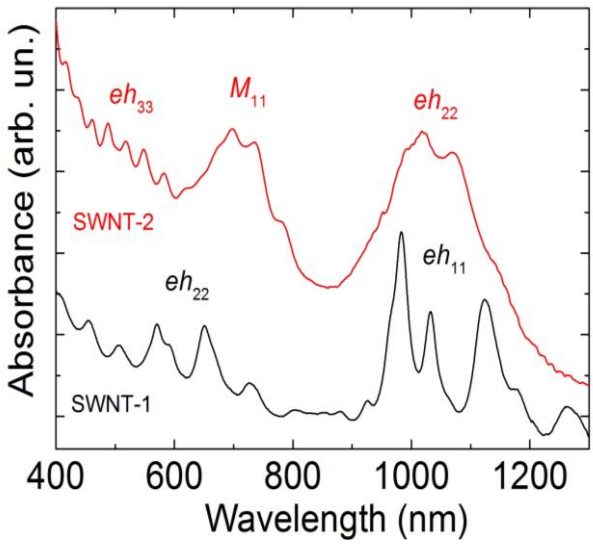

Figure S2: *Absorption spectra of SWNT-1 (black curve) and SWNT-2 (red curve). The labels eh_{11}, eh_{22}, eh_{33} and M_{11} refer to the first, second, third semiconducting and the first metallic excitonic transition, and are a guide to the eye, since overlap between different excitonic transitions exists [14, 15]. The spectra are normalized for a clear visualization.*

S3.1.1 Optical absorption spectroscopy of graphitic flakes

Fig. S3 report the absorption spectrum of the graphene dispersion. The peak at ~266 nm is a signature of the van Hove singularity in the graphene density of states [17]. OAS used to evaluate the concentration of graphitic material in dispersion, using the experimentally derived absorption coefficient of $1390 Lg^{-1}m^{-1}$ at 660nm [18, 19], we estimate concentration ~0.03mg/ml.

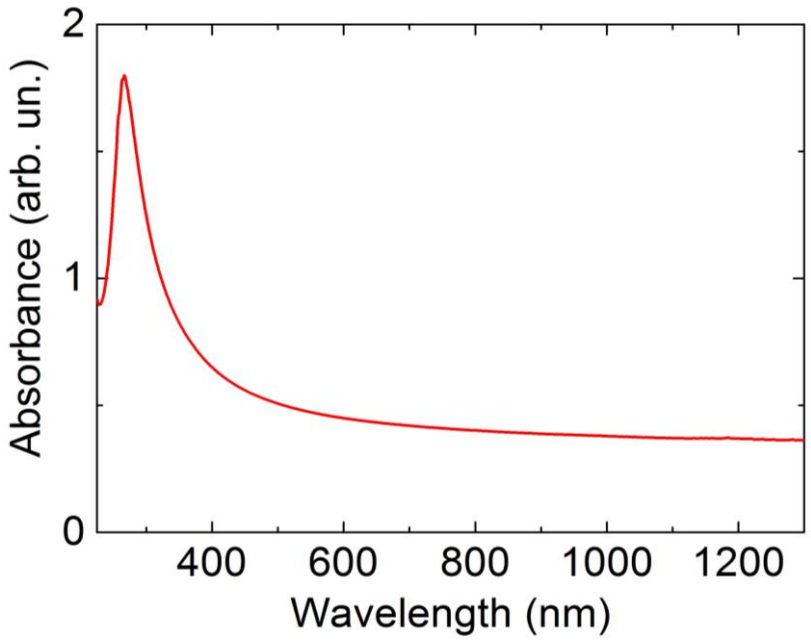

Figure S3: *Optical absorption spectrum of graphene dispersion in water with SDC surfactant.*

S3.2 Raman Spectroscopy

S3.2.1 Pristine SWNTs

Raman spectroscopy can be used to probe SWNTs within dispersions. In the low frequency region, the Radial Breathing Modes (RBMs) are observed [20]. Their position, Pos(*RBM*), is inversely related to SWNT diameter, *d* [21-23], as given by:

$$Pos(RBM) = \frac{C_1}{d} + C_2 .$$

Combining Pos(*RBM*), with excitation wavelength and the '*Kataura plot*' [15], it is, in principle, possible to derive the SWNT chirality [24, 25].

Matching the diameter with excitation wavelength in the Kataura plot also gives information on the semiconducting or metallic character. A variety of C_1 and C_2 were proposed for this relation [7, 8, 14, 26]. Here we use the $C_1 = 214.4\ \mathrm{cm^{-1}}$ nm and $C_2 = 18.7\ \mathrm{cm^{-1}}$, from Ref [27]. These were derived by plotting the resonance energy as a function of inverse RBM frequency without additional assumptions. We also validated our results by using the parameters proposed in Refs. [7, 10], [28].

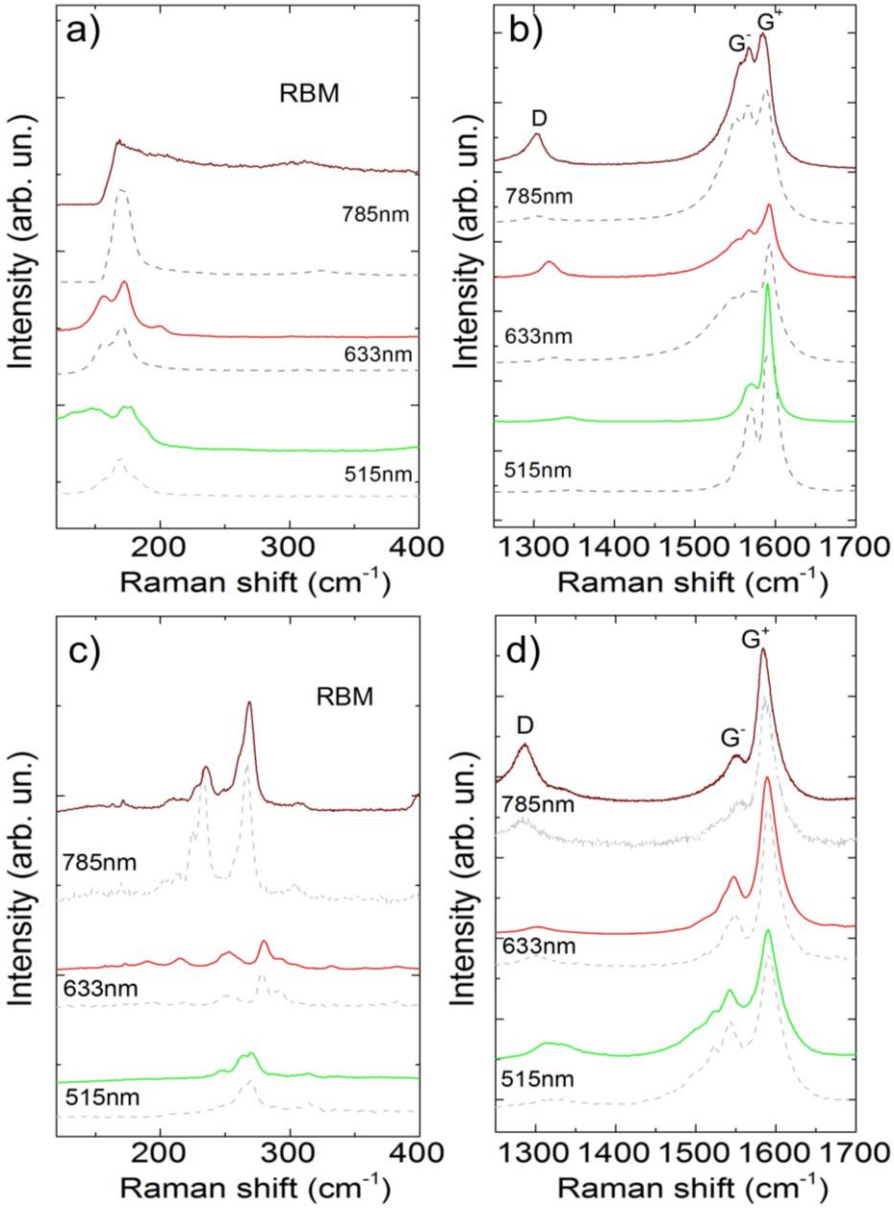

Figure S4: *Raman spectra of SWNTs at different excitation wavelengths: 514.5nm (green curve), 633nm (red curve) and 785nm (brown curve) (a) RBM and (b) G region for SWNT-1 and (c) RBM and (d) G region for SWNT-2. The Raman spectra of the starting materials (powders) are reported in light grey dashed lines below each curve for comparison.*

Raman spectroscopy also probes possible damage via the *D* peak [29]. The *D* peak is due to the breathing modes of sp^2 rings and requires a defect for its activation by double resonance (DR) [30, 31]. The typical Raman spectrum of SWNTs in the 1500-1600 cm^{-1} region consists of the G^+ and G^- bands. In s-SWNTs, they originate from the longitudinal (LO) and tangential (TO) modes, respectively, derived from the splitting of the E_{2g} phonon of graphene at the Brillouin zone centre [32-34]. The positions of the G^+ and G^- peaks, Pos(G^+), Pos(G^-), are diameter dependent and their separation increases with decreasing diameter [35, 36]. In m-SWNTs, the assignment of the G^+ and G^- bands is the opposite, and the FWHM of the G^- peak, FWHM(G^-), is larger and Pos(G^-) down-shifted with respect to the semiconducting counterpart [20, 37]. Thus, a wide, low frequency G^- is a fingerprint of m-SWNTs. On the other hand, the absence of such a feature does not necessarily imply that only s-SWNTs are present, but could signify that m-SWNTs are off-resonance.

Doping could also modify positions and FWHMs [38, 39]. In m-SWNTs, a Pos(G^-) blueshift, accompanied by a FWHM(G^-) decrease is observed with electron or hole doping[40, 41]. In s-SWNTs, doping upshifts Pos(G^+), but does not affect FWHM(G^+) [14, 23].

Thus, a large number of excitation wavelengths are necessary for a complete characterization of SWNTs [18, 42]. Nevertheless, useful information can be derived even with few excitation wavelengths.

Raman spectra are taken on both the starting materials (powder) and the dispersions, deposited by drop-casting onto an aluminium substrate to avoid any Raman background, with a Renishaw system at 514.5 nm (2.41 eV) 633 nm (1.96 eV) and 785 nm (1.58 eV), using a 100X objective and a less than 1 mW on the sample. The RBM detection is limited by the cut-off of the notch and edge filters. These are at 120, 100 and 110 cm^{-1} for 514, 633 and 785nm, respectively, limiting the detection to diameters up to ~1.9nm.

The RBM spectra of the SWNT-1 in Fig. S4(a) show a distribution, spanning the 175–370 cm^{-1} range both for the starting material and the dispersion, considering the peaks for the three excitation wavelengths. This RBM range corresponds to SWNTs with ~0.6–1.35nm diameter. Fig. S4(b) plots the spectra in the G region of SWNT-1. A weak D band is observed [I(D)/I(G)~0.13], indicating small number of defects [15, 16]. These defects could be induced by the ultrasonication process because the I(D)/I(G)~0.05 of the powder is lower with respect to the dispersion.

The RBM spectra of the SWNT-2 in Fig. S4(c) show a similar distribution but peaked at lower wavenumbers with respect to SWNT-1. Indeed, the RBMs span the 150–215 cm^{-1} range both for the starting material and the dispersion, considering the peaks for the three excitation wavelengths. This corresponds to SWNTs with ~1.05–1.65nm diameter. Fig. S4(d) plots the spectra in the G region of SWNT-1. A weak D band is also observed [I(D)/I(G)~0.18] for the SWNT-2 sample, indicating small number of defects [15, 16]. As for the SWNT-1, also in the case of the SWNT-2 sample, these defects could be induced by the ultrasonication process because the I(D)/I(G) ~0.08 of the powder is lower with respect to the one of the dispersion.

S3.3 Pristine flakes and dispersions

The ultracentrifuged dispersions are drop-cast onto a Si wafer with 300nm thermally grown SiO_2 (LDB Technologies Ltd.). These samples are then used for Raman measurements at 488, 514.5, and 633nm. The G peak dispersion is defined as Disp(G) =ΔPos(G)/$\Delta\lambda_L$, where λ_L is the laser excitation wavelength.

Fig. S5a plots a typical Raman spectrum of the flakes prepared on Si/SiO_2 substrates.

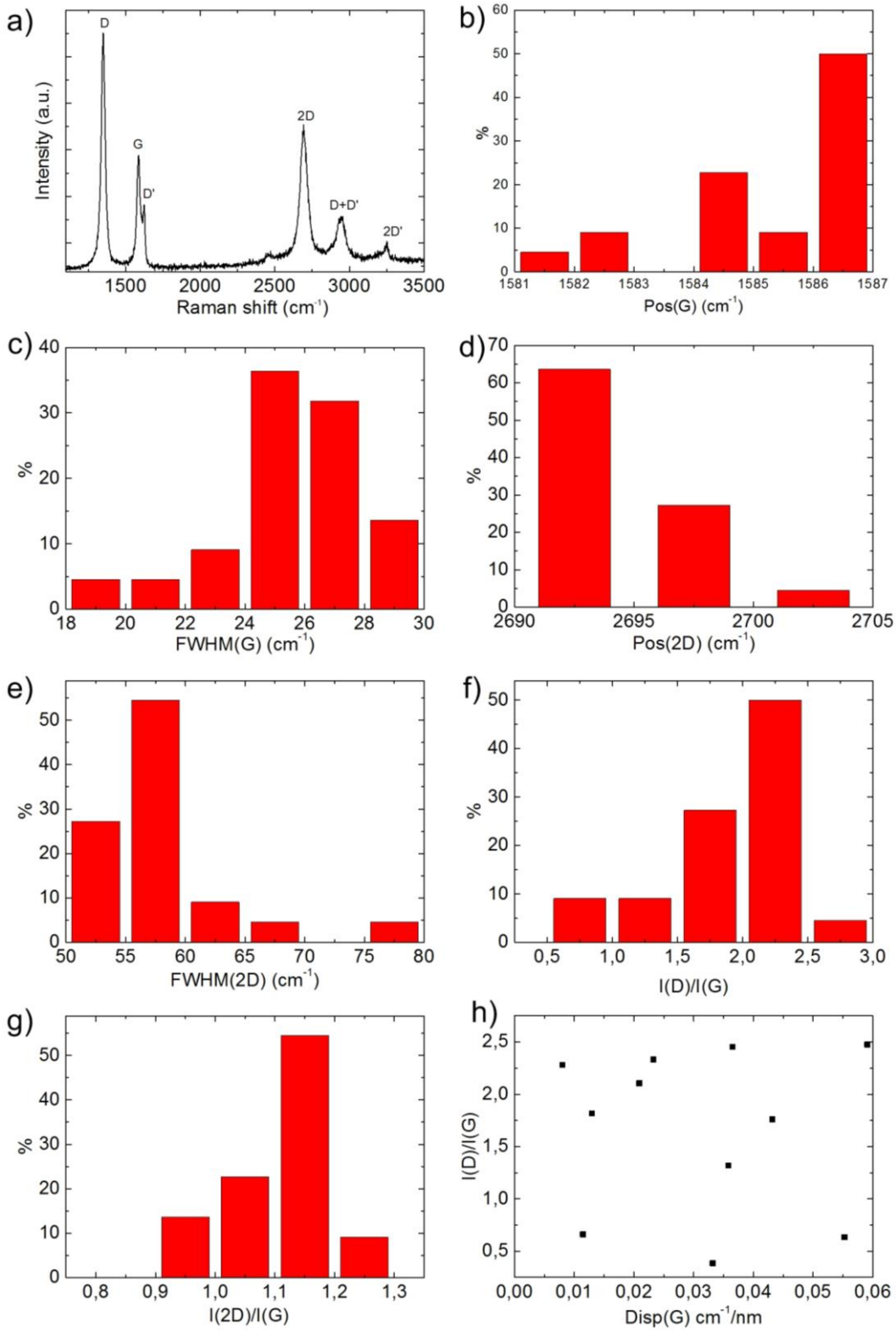

Figure S5: *a) Raman spectrum measured at 514.5nm excitation for a representative flake obtained via LPE of graphite. Distribution of b) Pos(2D), c) FWHM(2D), d) Pos(G), e) FWHM(G), f) I(D)/I(G), g) I(2D)/I(G), and h) distribution of I(D)/I(G) as a function of Disp(G).*

Besides the *G* and 2*D* peaks, this spectrum shows significant *D* and *D'* intensities and the combination mode *D*+*D*'. Statistical analysis of the spectra shows that Pos(*G*) (Fig. S5b) and FWHM(*G*) (Fig. S5c) are ~1582 and ~27cm^{-1}. Pos(2*D*) peaks ~2695cm^{-1} (Fig. S5d), while FWHM(2*D*) varies from

50 to 80cm^{-1} (Fig. S5e) with a peak at 57cm^{-1}. This is consistent with the samples being a combination of single- (SLG) and few-layer (FLG) graphene flakes. The Raman spectra show significant *D* and *D*' intensities, with the intensity ratio I(*D*)/I(*G*) having a maximum at 2.25 (Fig. S5f). This high I(*D*)/I(*G*) is attributed to the edges of our sub-micrometer flakes [43], rather than to the presence of a large amount of structural defects within the flakes. This observation is supported by the low Disp(*G*)<0.06 cm^{-1}/nm, much lower than what expected for disordered carbon [31]. Combining I(*D*)/I(*G*) with Disp(*G*) allows us to discriminate between disorder localized at the edges and disorder in the bulk. In the latter case, a higher I(*D*)/I(*G*) would correspond to higher Disp(*G*). Fig. S5h show that Disp(*G*) and I(*D*)/I(*G*) are not correlated, an indication that the major contribution to the *D* peak comes from the edges of the sample.

S.4 High Resolution Transmission Electron Microscopy

We analysed the samples with High Resolution Transmission Electron Microscopy (HR-TEM) using a FEI Tecnai F20 equipped with a Schottky emitter and operated at 200 keV, to determine the spatial distribution of the carbon nanoreinforcements. As shown in Fig. S6, carbonaceous material was observed between different fibers. Some crystalline graphitic material was observed in this area. However, from Fig. S6 it is not possible to observe the spatial ordering of individual CNTs or graphene inside the silk matrix. We note that Ref. [44] shows a very similar TEM image that also fails to provide evidence of the structure at nanoscale. The authors claim that "silk fibers show regions with highly ordered graphitic structures", but this ordering is not at all apparent in these images, nor is the deep integration of the nano-reinforcements in the silk amourphous matrix.

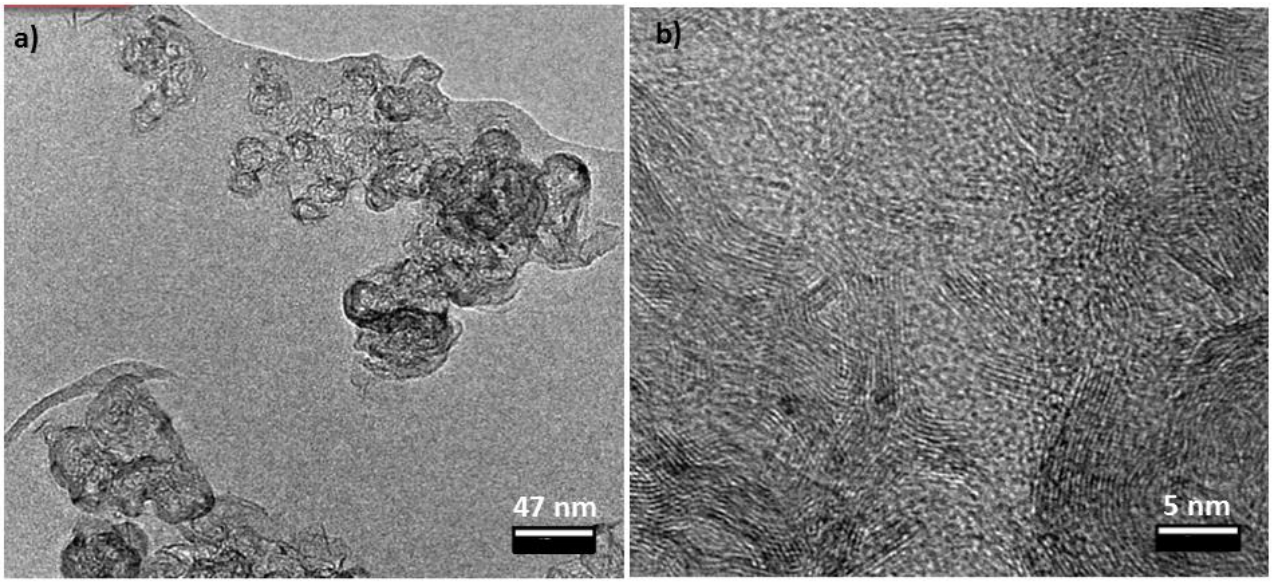

Figure S6: *HR-TEM images of the Graphene-reinforced silk at two different magnification levels.*

S.4 Nanotensile Tests

Nanotensile tests are performed under controlled laboratory conditions, since any change in temperature and humidity may affect the mechanical characteristics of the silk threads [45-48].

We monitor the experimental ambient conditions with a datalogger (EL-USB-2, Lascar Electronics): the air temperature and the relative humidity were recorded to be 22.8 ± 1.3 °C and 59.1 ± 5.0 %. We fixed silk fibre ends to 15 x 10 mm^2 "c" shaped cardboard holders, with double adhesive faces. These allowed the fibre to be suspended and mounted on the nanotensile testing machine, while maintaining its original tension without damage (Fig. S1g). The tests are conducted using a

nanotensile testing system (T150, Agilent, Santa Clara, USA), equipped with 500 mN maximum cell load. This can generate load-extension data with a load resolution of 50 nN and a 0.1nm displacement resolution. Cardboard holders are placed between the clamps. Once the holders are in place, the clamps are closed and one side of the holders is cut (Fig. S1h), leaving the fibre free between the clamps. We perform a continuous dynamic analysis of the silk by imposing an oscillating dynamic strain up to failure of the thread. We use a dynamic strain oscillation with a 20Hz frequency and a 0.1mN dynamic force amplitude, which enable mechanical properties to be determined continuously.

Typical stress-strain results for tests on various silk samples from the first 14 spiders are shown in Fig. S6 before and after exposure to the nanomaterial dispersions. The results are discussed in the Main Text.

We do not consider the effect of the nano-reinforcement volume fraction on the variation of density, since this is negligible for the considered volume fractions. In CNTs, the density ρ_{CNT} is between 1.3 and 1.4 g/cm^3 [49], similar to that of silk (1.34 g/cm^3 [50]). In the case of graphene, the density is close to that of graphite, i.e. ρ_G ~2.1 g/cm^3 [51-53], so the effect would be a slight increase in the overall density, and thus a small reduction in toughness modulus. For a very high f = 12% volume fraction of nano-reinforcements (usually the onset of agglomeration effects determining a deterioration of reinforcement properties occurs below 10%), there is an increase in density from ρ_S = 1.34 g/cm^3 for the pristine silk to ρ_{TOT} = 1.34 g/cm^3 and ρ_{TOT} = 1.43 g/cm^3 when CNTs or graphene are added (estimated using a simple rule of mixtures: $\rho_{TOT} = (1-f)\rho_S + f\rho_{CNT/G}$) corresponding to a 0.1% and 6.7% decrease of toughness modulus, respectively. Should CNTs and graphene additionally cause an increase in volume, this would contribute to a reduction in the overall density, therefore an increase of the toughness modulus. In any case, it is likely that these limited silk density variations would be smaller than the variations due to humidity mentioned in the main text [54], and can thus be neglected in the estimation of toughness moduli.

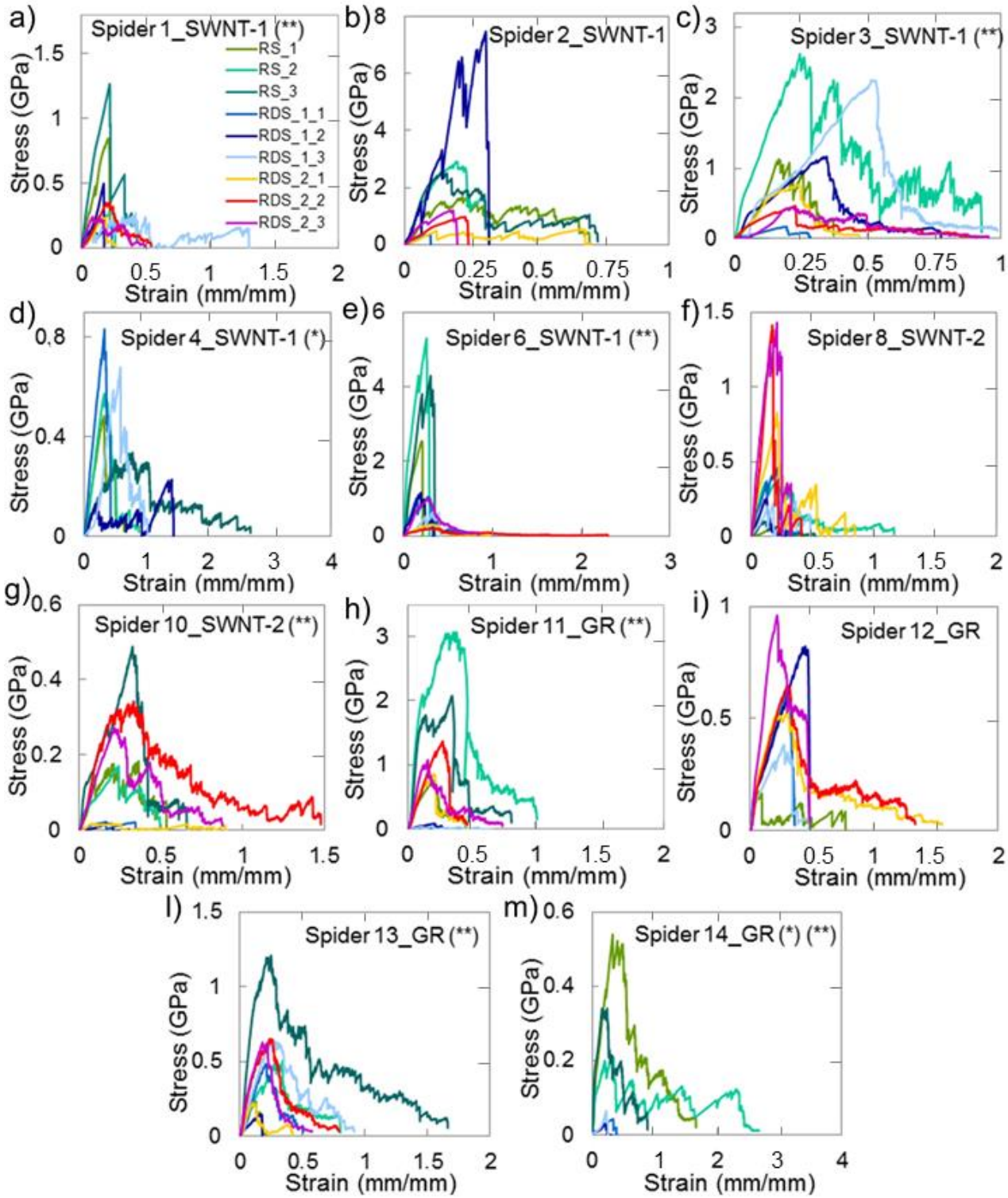

Figure S7: ***Stress-strain curves.*** *Stress-strain curves for silk produced by spiders after first (RDS_1) or second (RDS_2) ingestion of a-g) SWNT-1, SWNT-2 or h-m) graphene dispersions. The symbol "**" specifies enhanced mechanical properties (fracture strength and Young's modulus) after first collection. The symbol "*" specifies that spiders died before the second collection.*

A further collection of silk was carried out after a few days, but the corresponding samples did not display mechanical improvements with respect to the first or to RS, probably due to a physiological spider weakening during segregation, since neither additional food nor water were available during the experimental period, except SWNTs and graphene dispersions. Results are

reported in Table S2. In the cases marked with an asterisk in Figs. S6, the second collection was impossible since the corresponding spiders died. Note that spider 5 died after the first treated dragline silk collection, but was able to spin the silk with the maximum increment in mechanical performance, whereas spider 7 spun the silk with the highest absolute values and survived.

Spider n.	**Diameter (µm)**	**Number of threads**	**Reinforcement**	**Young's Modulus (GPa)**	**Ultimate Strain (mm/mm)**	**Fracture Strength (MPa)**	**Toughness Modulus (MPa)**
1	0.658±0.063	73	SWNT-1	2.8±1.1	0.423±0.142	275.1±65.5	55.3±26.9
2	0.410±0.023	27	SWNT-1	9.5±6.4	0.368±0.274	906.2±338.5	168.1±36.5
3	0.587±0.041	96	SWNT-1	5.1±0.7	0.753±0.258	562.5±178.5	157.4±10.4
4	-	-	SWNT-1	-	-	-	-
5	-	-	SWNT-1	-	-	-	-
6	1.071±0.140	160	SWNT-1	3.6±2.4	1.325±0.781	507.6±429.8	198.1±115.1
7	1.013±0.150	330	SWNT-2	3.6±1.8	0.818±0.386	473.1±273.0	195.6±163.6
8	0.180±0.020	58	SWNT-2	9.9±3.1	0.509±0.272	1222.8±341.9	196.9±41.1
9	-	-	SWNT-2	-	-	-	-
10	0.550±0.162	114	SWNT-2	1.4±0.9	1.055±0.325	211.2±171.0	99.6±95.8
11	0.413±0.056	89	GS	8.6±2.2	0.540±0.153	1102.5±255.7	241.4±69.4
12	0.640±0.056	240	GS	5.6±2.1	1.082±0.554	716.7±216.2	286.7±24.4
13	0.586±0.074	144	GS	5.6±1.4	0.577±0.171	499.6±240.3	125.7±85.8
14	-	-	GS	-	-	-	-
15	0.533±0.118	153	GS	2.5±1.8	1.138±0.465	216.9±132.0	110.4±101.4

Table S2. *Mechanical properties (average values) of the second collection of silk samples produced after exposure of the spiders to the SWNT and graphene dispersions. 26% of the spiders died between the first and second collections, thus the corresponding data are absent.*

Table S3 shows average values for similar types of silk found in the literature [55-57], and their corresponding standard deviations, showing that uncertainties are in many cases comparable to those in this work.

Source	Young's Modulus (GPa)	Ultimate Strain (mm/mm)	Fracture Strength (MPa)	Toughness Modulus (MPa)
This work	10.73±4.84	0.70±0.35	1082.94±504.48	313.70±204.78
Ref [55]	11.11±3.67	0.22±0.05	1095.17±296.12	125.49±42.87
Ref [56]	10.08±1.32	0.30±0.02	986.5±107.25	194.8±23.2
Ref [57]	11.50±3.85	0.62±0.15	1634.00±323.67	217.00±84.33

Table S3. *Average mechanical properties for spider silks in the literature, compared to those found in this work*

S.6 Statistical data analysis and numerical HFBM simulations

Analysis of experimental data discussed in the Main Text and in the "Nanotensile Tests" Section indicates that there is a large statistical spread in the measured mechanical properties of RS as well as CNTS and GS. In the case of GS and CNTS it is not possible to compare samples from different spiders, since their concentrations are different, so that samples belong to different statistical populations. On the RS samples, a statistical analysis of the experimental data is performed to verify if they belong to the same statistical population, despite deriving from different spider species. To do this, we adopt the Weibull distribution, which is widely used in fracture mechanics and particularly suitable to describe dispersion of mechanical properties [58], and fit the data relative to RS. The resulting 2-parameter Weibull distributions are shown in Fig. S8. The parameters for the corresponding Weibull distributions of RS are summarized in Table S4. Scale parameters are indicative of average values, whilst shape parameters are correlated to the dispersion of the distributions.

RS	Mean	STD	Shape parameter	Scale parameter
Young's Modulus (GPa)	10.73	10.43	1.19	11.06
Strength (MPa)	1082.94	1082.94	1.27	1126.55
Ultimate Strain	0.70	0.40	2.09	0.79

Table S4. *Mean Young's modulus, strength and ultimate strain of RS and corresponding Weibull parameters.*

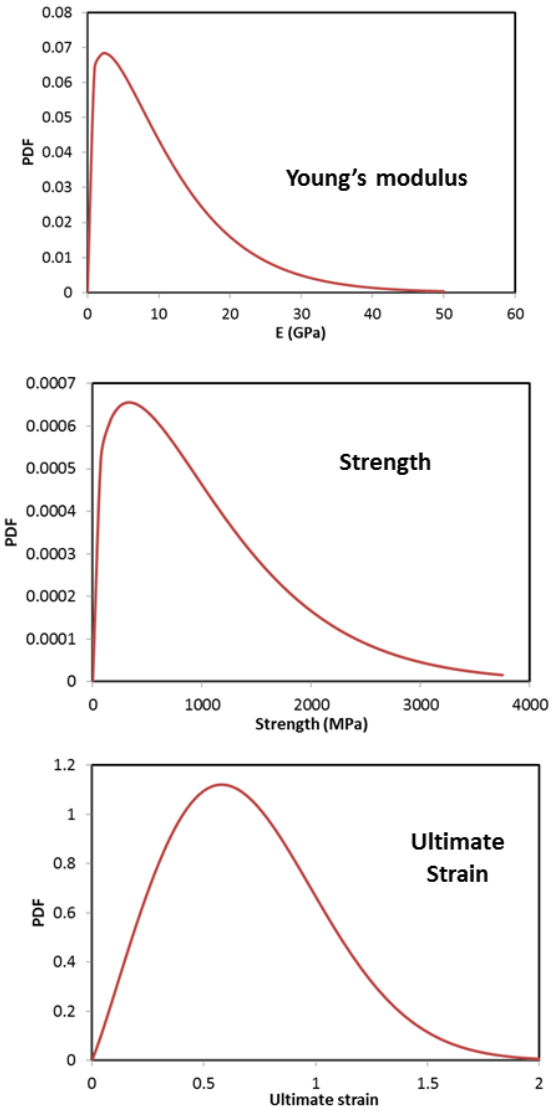

Figure S8: Weibull fits for mechanical data on RS. *Weibull fits (Probability Density Functions, PDF) on experimental data for a) Young's modulus, b) strength and c) ultimate strain of RS.*

Numerical simulations of deformation and fracture are performed using the Hierarchical Fibre Bundle Model (HFBM) [59], whereby the macroscopic fibres are modelled as networks of microfibres and/or reinforcements arranged in parallel and in series, subjected to uniaxial tension. The microfibers are treated as elastic springs with statistically distributed fracture strengths, according to measured or known input parameters for the constituents (i.e. the experimentally-determined Weibull parameters from Table S.4 for the silk, and known mechanical properties from the literature for CNTs and graphene). As in all fibre bundle models, interfacial interaction between threads in a bundle is neglected, apart from load redistribution after failure of a single thread. This is akin to neglecting shear effects. However, this was shown to be a reasonable approximation in the case of tensile testing of this type of fibre as discussed in Ref. [59]. The statistical distribution of strengths in the fibres forming a bundle reproduces correctly the fracture behaviour of individual macroscopic fibres due to microcracking. Simulations are carried out in a multiscale procedure, from single SWNT (diameter ~1.5 nm, length ~250 nm) scale to the macroscopic scale of specimens used in experimental tests (diameter ~ 5 μm, length ~1 cm). The silk/silk and silk/nanoparticle interaction (in terms of load redistribution) is modelled only at the lowest hierarchical level. At higher hierarchical levels the fibre

is considered as consisting of homogeneous threads. The typical number of fibres used in upper hierarchical level simulations is 10^3 (this is to be interpreted as a discretization parameter used to simulate damage progression). We consider typical values for SWNT and graphene properties, respectively: Young's modulus $E_{CNT} = E_{SLG} = 1$ TPa [60, 61] and strength $\sigma_{CNT} = 45$ GPa [60], $\sigma_{SLG} = 130$ GPa [61]. The experimentally obtained RS Weibull distributions are used as input properties. Figure 9 shows typical simulations results for stress-strain curves with different SWNT volume fractions V_f, assuming a uniform SWNT dispersion at the lowest hierarchical simulation level (i.e. that at which individual SWNTs coincide with springs in the bundle). Figure S10 indicates that there is an overall strength and modulus increase with increasing V_f, while there is a corresponding ultimate strain reduction. As a reference, comparisons are made with rules of mixtures (RM, see Fig. S10). As expected, HFBM predictions for strength lie between direct and inverse rule of mixtures predictions.

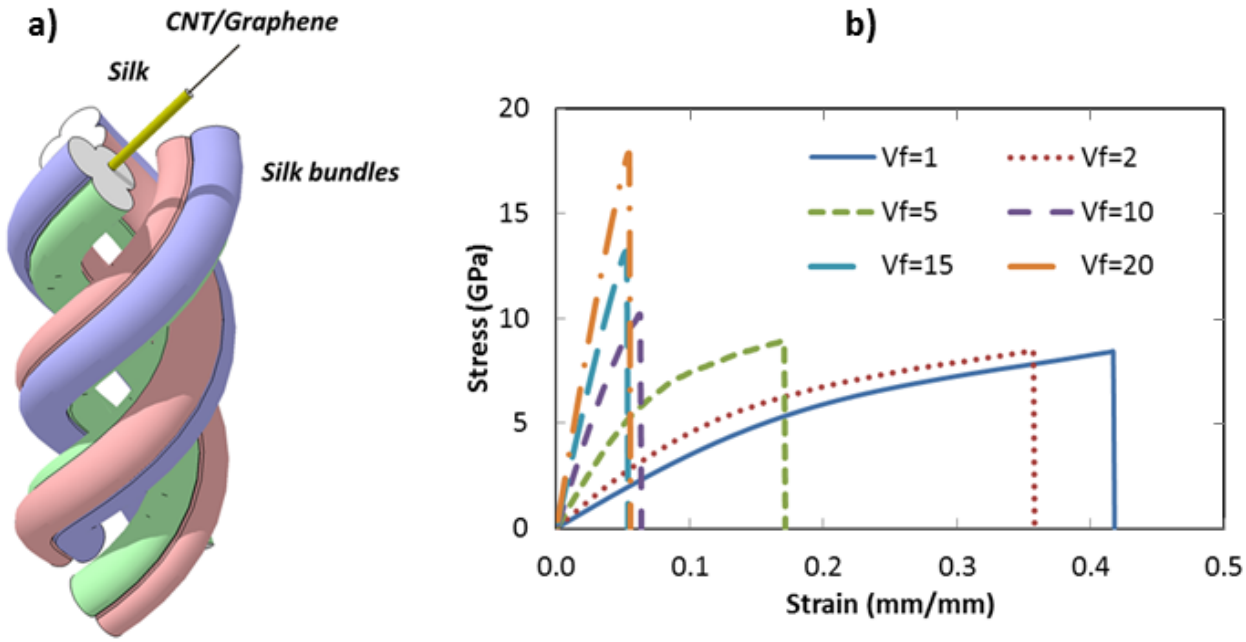

Figure S9: HFBM simulations. *a) Schematic of the model and b) examples of stress-strain results for different volume ratios.*

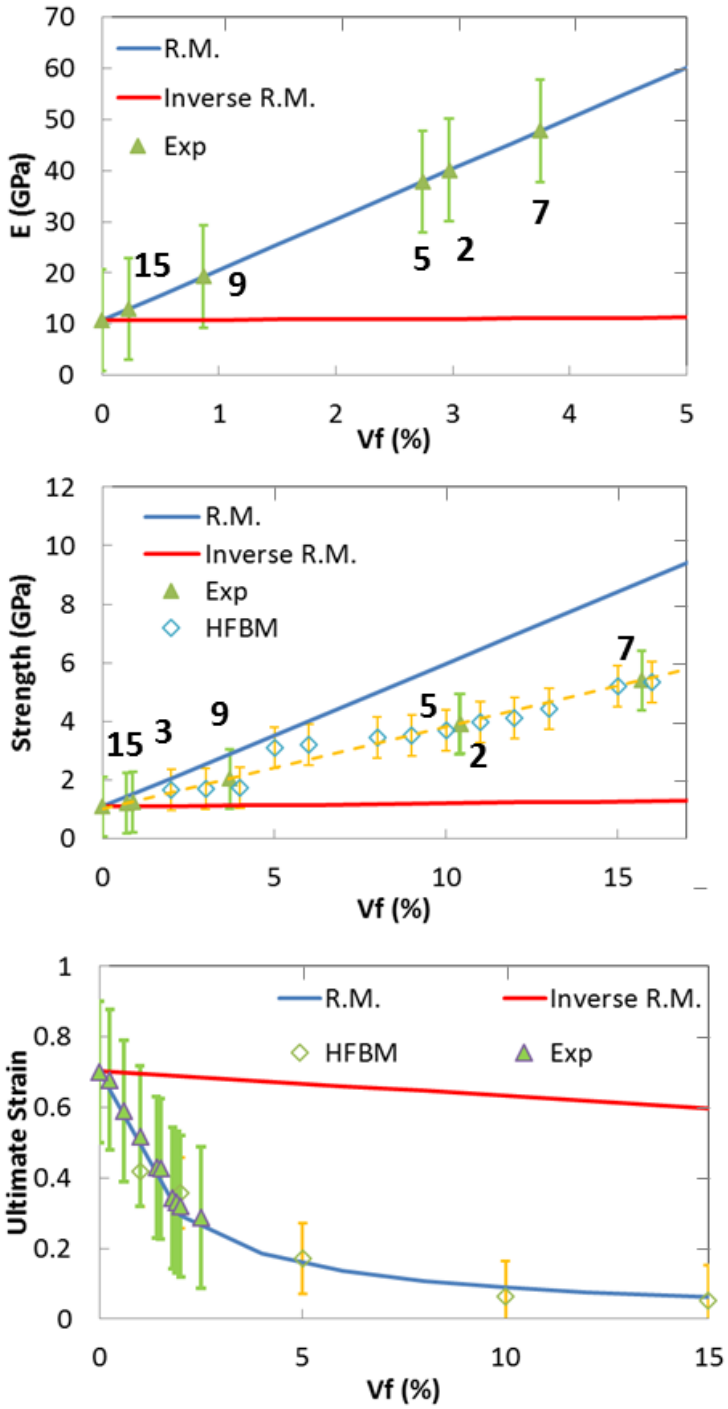

Figure S10: Comparison of experiments with HFBM simulations and direct or indirect rules of mixture (RM). *RM predictions are shown as blue lines (direct RM law, taken as an upper bound for predictions) and as red lines (inverse RM law, taken as lower bound for the predictions). Experimental data are plotted as green triangles, with corresponding sample numbers, and with error bars corresponding to the experimental standard deviations (with V_f values estimated from RM or HFBM, as explained below), while HFBM data are shown as hollow diamonds, with error bars corresponding to uncertainties due to statistical variations in the simulations. Only experimental data compatible with a rule of mixtures V_f estimation. i.e., with improved properties with respect to RS, are shown. These values are "effective" in the sense that they consider also the expected sik modification around the nanoparticles. For ab initio simulations see [62].*

S.7 Atomistic simulations of the mechanical properties of spider silk

We perform classical molecular dynamics simulations in order to obtain a qualitative understanding of the CNT and graphene's interaction with the silk proteins. We generate a model of the MASP1 spider silk protein as in Refs. [63, 64]. We use the CHARMM27 force field implemented in GROMACS (v. 4.6.7) [65] to model the molecular interactions. In order to have a charge-neutral system we substitute arginine with glutamine (the neutral aminoacid with the closest structure). The final aminoacid sequence of the fiber is:

`GGAGQGGYGGLGSQGAGQGGLGGQGAGAAAAAAGGAGQGGYGGLGSQGAGQGGLGGGAG.`

From this, we generate a fully extended MASP1 fiber using OpenBabel (v. 2.3.2) [66], from which a bundle of 15 fibers (aligned along the x direction) is prepared by translation and inversion to facilitate the formation of the central ordered region. Initially, the fibers are kept separate (1 to 2 nm), so as to be able to insert graphene or CNTs in the amorphous region and to prevent the disruption of the crystalline poly-alanine core. In simulations, we used capped nanotubes with a diameter of ~0.8 nm, which is comparable to the experimental value of ~0.6–1.35 nm for SWNT-1 and ~1.05–1.65 nm for SWNT-2. The length of 1.8 nm of the CNTs in simulations was chosen so to have dimensions smaller/comparable to the disordered part of the modelled fiber (around 3.5 nm over a total fiber length of about 11 nm, see Fig. S11 a). This is much smaller than the experimental value. Within the accuracy of the chosen silk model [63, 64], the interaction between CNTs and fiber scales linearly with the CNT length since the adopted interatomic potential is pair-wise and the CNTs can be considered essentially one-dimensional. Based on this argument, the interaction per unit cell can be considered nearly invariant with the CNT length, and in our investigation, we take a CNT length that reproduces a characteristic CNT volume fraction of f = 12% (see macroscopic simulations in section S6, where the CNT length is taken to be 250 nm). The same arguments apply for the graphene flakes, which are thus modeled as square-shaped sheets with a lateral dimensions of ~2 nm (much smaller than the experimental dimensions). CNTs and graphene are modeled using the DREIDING force field to account for their flexibility and the interaction with the protein's atoms. This initial configuration is equilibrated using the following procedure: after a conjugate-gradient local minimization, we perform 150 ps molecular dynamics (MD) at low (T=10 K) temperature, similar to the procedure developed in Refs. [63, 64], adding a harmonic force with constant k = 100 kJ mol-1 nm-2 in the yz plane to drive the fiber formation. The choice of low temperature for this equilibration part is dictated by the need to keep the well-ordered central region, responsible for the rigid beta-sheet structure of the actual protein [63, 64]. The resulting configurations are used as starting points for 500 ps MD runs at T=300 K. All calculations are performed using the Generalized-Born Surface-Accessible implicit solvent model [67] to mimic a wet environment surrounding the silk assuming a continuum model of water. Fig.S11a shows the spider-silk protein, with a β-sheet rich crystalline region and an α-helix-rich amorphous region, in good agreement with Refs. [63, 64]. Graphene does not qualitatively change this, Fig.S11b. The simulations show that graphene tends to slide out of the protein, while remaining anchored to the amorphous part. Fig.S11c indicates that CNTs tend to remain trapped within the amorphous region with two structural consequences: a slight disruption of the crystalline order of the β-sheet region, and a tendency to increase the amount of disorder in the α-helix.

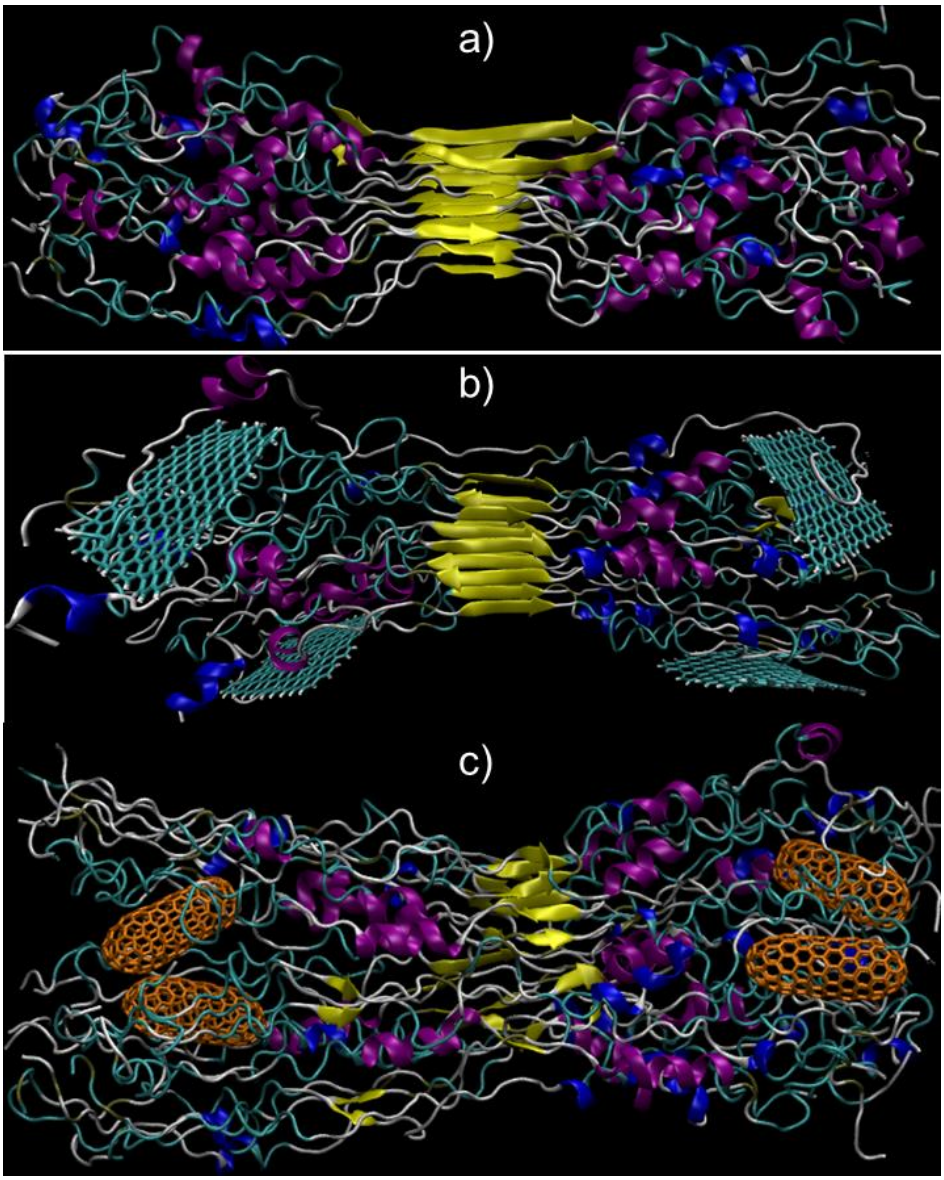

Figure S11: *Representative structures of (a) pristine MASP1 bundle, and after (b) graphene and (c) CNT incorporation. Beta sheet: yellow, alpha helix: purple, 3-10 helix: blue. Graphene is represented in green and CNTs in orange.*

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