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ARTICLE TYPE

# Preparation of hexagonal GeO<sub>2</sub> particles with particle size and crystallinity controlled by peptides, silk and silk-peptide chimeras

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We demonstrate the use of silk based proteins to control the particle/crystallite size during GeO<sub>2</sub> formation, using a bio-mimetic approach at circumneutral pH and ambient temperature. Multicrystalline GeO<sub>2</sub> was prepared from germanium tetraethoxide (TEOG) in the presence of different silk-based proteins: Bombyx mori silk (native silk) and two chimeric proteins prepared by linking a germania binding peptide (Ge28: HATGTHGLSLSH) with Bombyx mori silk via chemical coupling at different peptide loadings (silk-Ge28 10% and silk-Ge28 50%). The mineralisation activity of the silk-based proteins was compared with that of peptide Ge28 as a control system. GeO<sub>2</sub> mineralisation was investigated in water and in citric acid/bis-tris propane buffer at pH 6. Morphology, particle size, crystallinity, water and organic content of the materials obtained were analysed to study the effect of added biomolecules and mineralisation environment on material properties. In the presence of silk additives well-defined cube-shape hybrid materials composed of hexagonal germania and up to ca. 5 wt% organic content were obtained. The cubic particles ranged from 0.4 to 1.4 μm in size and were composed of crystalline domains in the range 35-106 nm depending on the additive used and synthesis conditions. The organic material incorporated in the mineral did not appear to affect the unit cell dimensions. The silk and chimeric proteins in water promote material formation and crystal growth, possibly via an effective ion-channelling mechanism. The germania binding peptide alone did not have any significant effect on reaction rate, yield or the material's properties compared to the blank. Interestingly, the peptide content in the silk chimeras tested did not affect mineralisation. The presence of buffer inhibited mineral condensation rate and yield. The use of silk-based biomolecules allows control of crystallite/particle size of hybrid materials opening up opportunities for bio-inspired approaches to be applied for the synthesis of functional germania based devices and materials.

## Introduction

The development of organic-inorganic composites with superior functional properties is an important aspect of modern materials research. In the design and preparation of such materials we can take inspiration from biominerals where the biomolecule (e.g. a protein) acts as a template or guides mineral formation, often controlling the mineral's growth, resulting in materials with superior properties.<sup>1-3</sup> The bottom-up biomimetic approach to the formation of materials is a promising method, that achieves high level of control under mild synthetic conditions and has been successfully applied to silica and other minerals,<sup>4-7</sup> using peptides and macromolecules to promote/ template the formation of specific nanostructures.

Germanium dioxide, or germania (GeO<sub>2</sub>), is a chemical analogue of silica with two main polymorphs: hexagonal (trigonal) quartz-like structure; and tetragonal (cubic) rutile-like structure.<sup>8</sup> GeO<sub>2</sub> nanostructures have unique and attractive physicochemical and optical properties for applications in optical, electronic and optoelectronic devices.<sup>9-11</sup> Hexagonal GeO<sub>2</sub> is commonly prepared by high temperature synthetic routes using germanium alkoxides<sup>12,13</sup> or Ge powder<sup>14</sup> as precursors, or under milder conditions (room temperature), from GeCl<sub>4</sub><sup>15</sup> and

germanium tetraethoxide<sup>16</sup> precursors by using a reverse micelle system. The discovery that marine organisms, such as diatoms and sponges, have the ability to incorporate inorganic Ge into their skeleton has reinitiated an interest in its potential role in biomineralisation processes.<sup>17</sup>

GeO<sub>2</sub> mineralisation from an alkoxide precursor has been previously studied in the presence of several bio-additives. GeO<sub>2</sub> mineralisation in the presence of self-assembled synthetic diacetylene phospholipids<sup>18</sup> and poly (allylamine hydrochloride)<sup>19</sup> gave amorphous GeO<sub>2</sub> nanostructures. An amphiphilic peptide able to form micelles in solution was used to template amorphous GeO<sub>2</sub> hollow spheres up to 600 nm in diameter.<sup>2</sup> Basic amino acids such as lysine were shown to yield crystalline hexagonal germania,<sup>20</sup> while peptides identified by biopanning against crystalline germania, have been shown to promote formation of amorphous germania.<sup>21,22</sup> Although the interaction between the anionic germanate species resulting from the hydrolysis of precursor and the cationic groups from the additives were proposed to be responsible for mineralisation, the exact role of the additives in the hydrolysis/condensation mechanism of mineral formation from alkoxides remains unclear.

In our study we used silk based proteins as bio-additives in the

hydrolysis/condensation medium for GeO<sub>2</sub> precipitation. Silks are fibrous proteins produced by spiders or silkworms and characterised by a unique range of properties: mechanical strength, smooth surface, flexibility, biocompatibility and biodegradability, that make them attractive for biological and tissue engineering applications like bone regeneration.<sup>23-26</sup> Furthermore, silk is also optically active, a required property for the development of biodegradable optical fibers, biosensors or biodetectors.<sup>27,28</sup> Silk proteins have been previously used as a scaffold for the bio-mimetic synthesis of silica,<sup>29</sup> silver<sup>30</sup> or gold<sup>31</sup> nanostructures. Silk can be modified to include additional functionality such as active sites for mineralisation (i.e.: from peptides)<sup>32</sup> therefore it presents an ideal platform for the development of organic-inorganic composites. A chemical method to achieve peptide grafting on silk involves the coupling of peptides to side chains containing carboxyl groups by diazotation followed by EDC/NHS cross-linking, allowing the theoretical loading of up to 365 peptide per silk molecule.<sup>33</sup> Silica-silk composite materials were also prepared with the chimeric scaffold being used to direct the precipitation of silica and control both morphology and particle size distribution of the final material.<sup>32-34</sup>

In the present study, we investigate the bio-mimetic mineralisation of germania in controlled pH and in the presence of silk proteins and chimeras based on *Bombyx mori* silk coupled with a germania binding peptide Ge28 (HATGTHGLSLSH),<sup>22</sup> which is likely to recognise the GeO<sub>2</sub> surface via Histidine and hydroxyl groups.<sup>22</sup> Our results show that polycrystalline hexagonal GeO<sub>2</sub> of cubic morphology with particles of up to 1.4 μm side length could be produced under mild conditions of pH, temperature and pressure using these bio-additives. The presence and nature of the additives and the solution reaction conditions were found to affect the precipitation yield, materials' properties and composition. In particular it was found that the size of crystallite domains and particle size were strongly dependent on the nature of the biomolecule used and on the presence of the buffer. The ability to control hexagonal germania particle size is important as this may lead to the enhancement of materials properties, like the charge retention capacity, when using germania as the anode in lithium batteries.<sup>35</sup>

## Results & discussion

### Reaction conditions

Germania mineralisation was carried out at circumneutral pH (5.93±0.3 by citric acid/bis-tris propane buffer) in the presence of different biomolecules in the hydrolysis/condensation media at a concentration of 1 mg/ml. Biomolecules studied were: peptide Ge28 (HATGTHGLSLSH); native silk protein; and two peptide-silk chimeric proteins of different peptide content (silk-Ge28 10% and silk-Ge28 50%) obtained by chemical coupling.<sup>33</sup> The chosen ratio silk/Ge28 in the present work covered up to 10% and 50% of the silk active sites (1:36 and 1:182 silk:peptide ratio, respectively). Higher silk modification rates were expected to lead to a conformational change of the protein structure,<sup>33</sup> and thus were not investigated. The properties of the additives in solution for both condensation systems are reported in Table 1. Native silk protein is composed of a heavy chain consisting of

5263 amino acids and of a light chain consisting of 262 amino acids in a 1:1 ratio.<sup>36,37</sup>

**Table 1** Solution properties of additives used in the bio-inspired mineralisation of germania in water and citric acid/bis-tris propane at ca. pH6. Theoretical pI and pH of 1mg/ml additive solution.

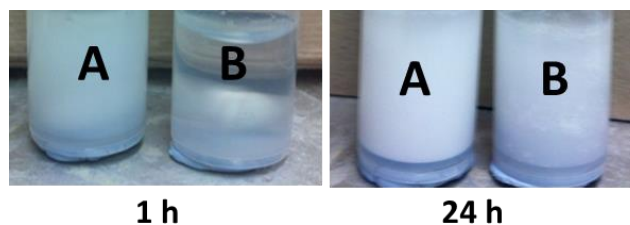
Additive	Theoretical pI <sup>1</sup>	pH	
		water	in buffer
Ge28	7.02	3.28±0.11	5.85±0.01
Native silk	4.39(HC) 5.23(LC) <sup>2</sup>	7.40±0.81	5.99±0.01
silk-Ge28 10%wt	n.a.	6.15±0.10	5.97±0.10
silk-Ge28 50%wt	n.a.	6.26±0.02	5.95±0.10.

<sup>1</sup> Calculated by ExPASy Compute pI/Mw tool. <sup>2</sup>HC: Heavy chain, LC: Light chain, n.a.: not available.

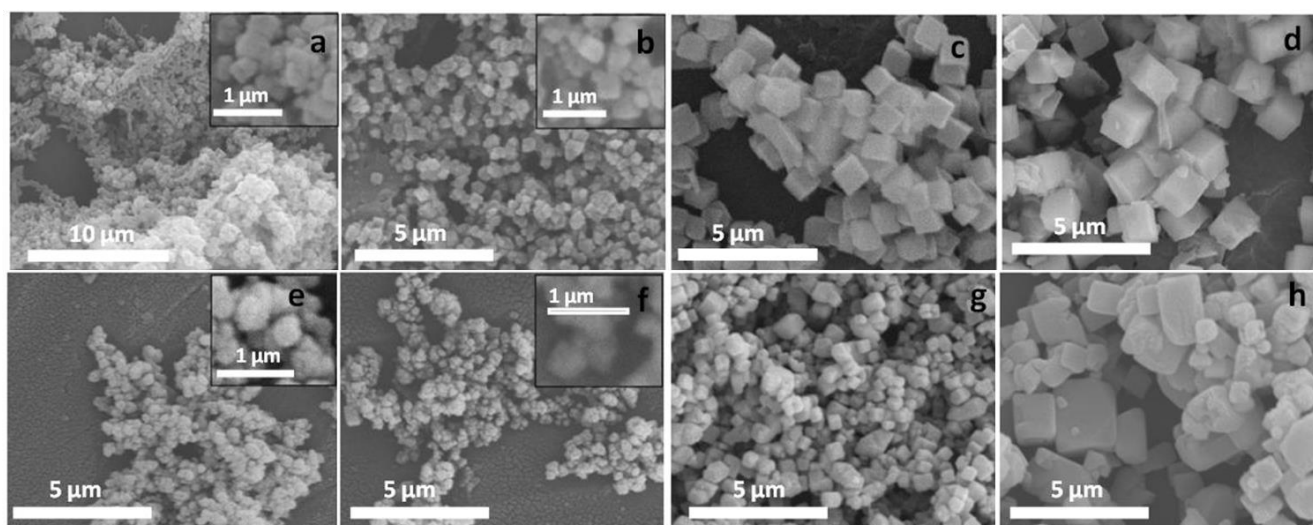
Heavy and light chain have an isoelectric point (pI) of 4.39 and 5.23, respectively, making native silk a slightly acidic protein. (Table 1). The citric acid/bis-tris propane buffer was used to allow comparative results for future studies over a wide pH range. However, since the presence of multivalent ions in the mineralisation system has been previously shown to affect the morphology of minerals formed in the presence of peptides<sup>38</sup> or polyallylamine,<sup>39</sup> a simplified water only system was also investigated. The presence of buffer in the condensation system affected the rate of precipitation as shown in Fig. 1. When the germania precursor (TEOG) was added to the biomolecule solutions in water, turbidity due to germania precipitation appeared in less than an hour, while under buffered conditions ca. 24 hours were needed for a white precipitate to appear.

### Characterisation of materials

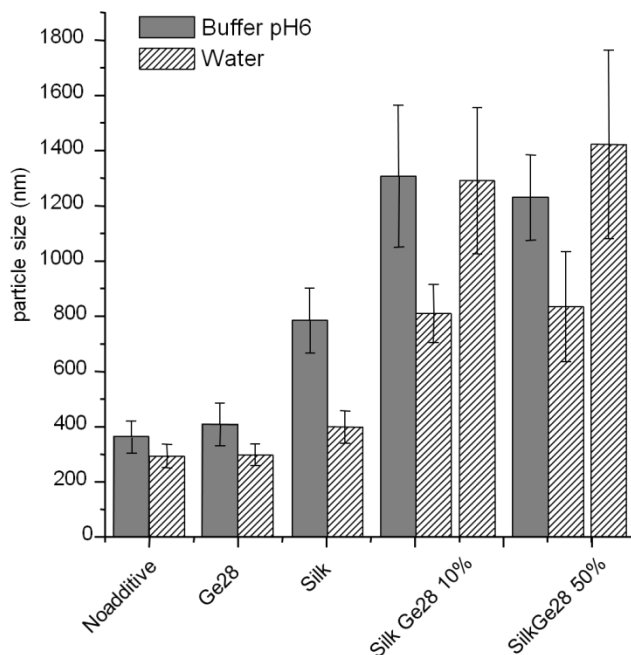
SEM/ EDX was used to investigate the morphology and elemental composition of precipitates after 48 hours condensation, respectively. All precipitated materials contained germanium and oxygen (Fig. S1, ESI†). The nature of the additive and the composition of the condensation system impacted both the morphology and size of the obtained materials. During mineralisation a small quantity of bio-additive was incorporated in the mineral yielding hybrid materials. The presence of Silk based additives generally led to aggregates of particles with a well-defined cubic shape from both buffered and water systems. In the presence of peptide Ge28 aggregated particles of less defined cubic shape, comparable to those obtained in the control (no additive) were obtained. A significant variation in the size of the cubic particles was observed depending on the nature of the additive present in the mineralisation system, whereas the presence of the buffer although generally resulting in larger particles



**Fig.1** Appearance of reaction vials after 1 hour and 24 hours for reactions conducted in water (A) and buffer at pH 6 (B).



**Fig.2** SEM images of materials resulting from mineralisation of 0.2 M TEOG in the presence of different additives and mineralisation environment. In the presence of citrate/bis-tris buffer (*ca.* pH 6): No additive (a), Ge28 (b), native silk (c) and silk-Ge28 50% chemical chimera (d). In water: No additive (e); Ge28 (f); native silk (g) and silk-Ge28 50% chemical chimera (h). All additives were used at a concentration of 1mg/ml.



**Fig.3** The effect of synthesis conditions and additive identity on  $\text{GeO}_2$  cube side length (nm). For silk-Ge28 chimeras two main particle size populations are reported (22% at *ca.* 800 nm diameter and 78% at *ca.* 1200-1400 nm diameter).

larger particles had a smaller impact on size (Fig 2, Fig.3). Bigger  $\text{GeO}_2$  cubes were obtained in the presence of the pH 6 buffered silk-Ge28 chimeras (side length *ca.* 1200-1300 nm). There was no significant difference in particle sizes between the 10% and 50% loaded silk-Ge28 germania products. Two statistically distinct particle size populations ( $\alpha=0.05$ ) were identified for materials prepared in the presence of silk-Ge28 chimeras in water; a minority of particles at *ca.* 800 nm diameter (22%) and a majority (78%) of particles at *ca.* 1200-1400 nm

diameter.

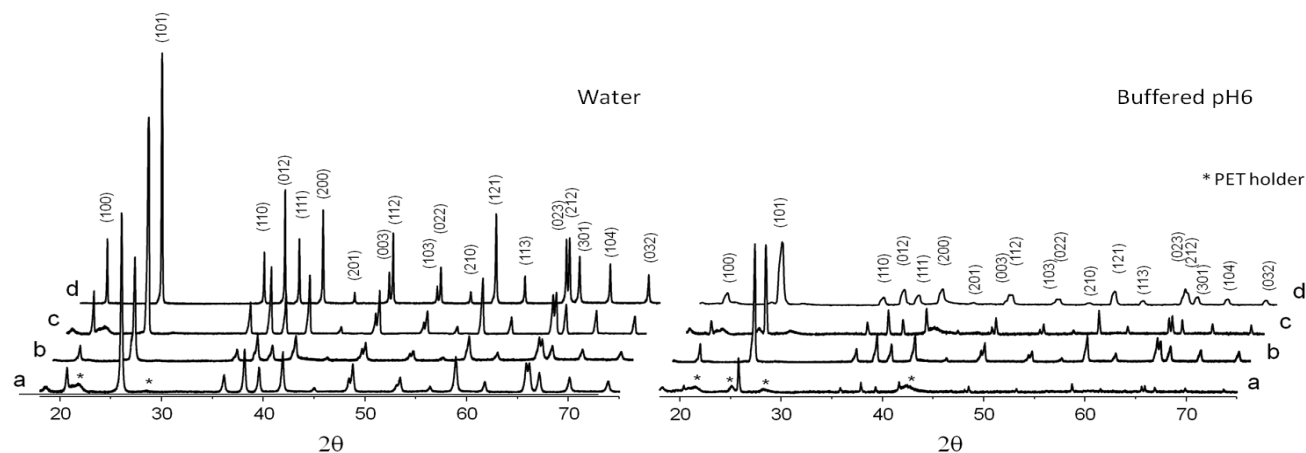
All precipitates were crystalline as shown by XRD analysis, Fig. 4. The diffraction patterns were indexed to pure hexagonal ( $\alpha$ -quartz phase, trigonal system) with space group  $P3_121$  ( $N^\circ$  152) and lattice constants of  $a=b=4.9823424 \text{ \AA}$ ,  $c=5.642891 \text{ \AA}$ .<sup>40</sup> The Scherrer equation was used to estimate the crystallite domain sizes of the materials (Table 2). Crystallite domain ranged from 47 to 106 nm, indicating that the germania cubic particles were multicrystalline. There was a strong dependence of the crystallite sizes on the nature of additive present in the mineralisation system. Similarly to what was observed for particle size, the presence of Ge28 did not affect the materials' properties compared to the control, while the silk protein and chimeras led to bigger crystals. The biggest crystallite sizes obtained were for materials generated in the presence of silk-Ge28 chimeras in water. Buffer had little effect on crystallite size in the presence of native silk, while it produced a decrease in crystallite size when the silk-peptide chimeras were used as additives.

TGA was used to quantify the organic content and the hydration in the precipitates, (Fig. 6) which were found to be lower than 5% and 1%, respectively for all precipitates, with products synthesised in water. Materials prepared in the presence of native silk in buffer showed the highest organic content (4.5%). The organic content in these materials can be attributed mainly to the silk protein. The percentage of organic matter in the materials obtained from the buffered control (no additive) corresponded to *ca.* 0.3% can be attributed to residual buffer not being removed during washing. To verify if the organic matter is intercalated in the crystal structure of the hybrid materials, we assessed the lattice constants using Rietveld analysis. The small amount of organic material caused no perceptible change in the lattice constants for the hybrid materials obtained in the presence of silk additives from those of pure hexagonal germania (Table 2) indicating that organic material was not intercalated in the crystal structure.

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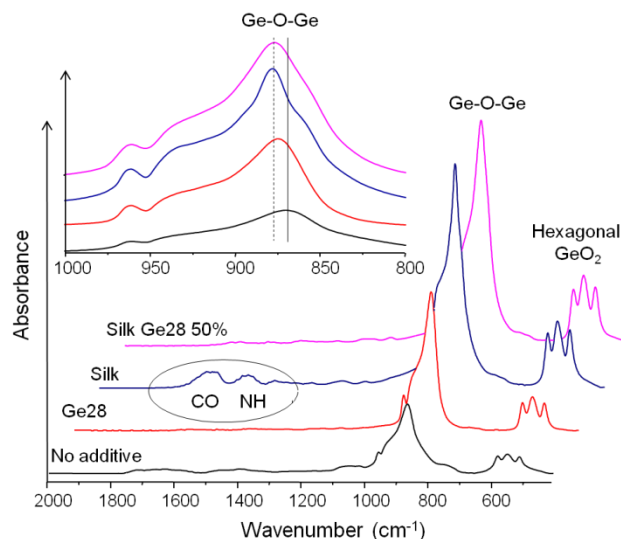


**Fig. 4** X-Ray diffraction patterns for materials obtained in the presence of 1mg/ml additive in water and buffer at pH 6: (a) blank, (b) Ge28, (c) native silk and (d) silk-Ge28 50%. All patterns are reported on the same scale. Asterisks indicate peaks from the PET sample holder.

**Table 2** Crystallite domain sizes of the various samples, lattice constants and particle size.

Additive	Condensation system	crystallite size (nm) <sup>a</sup>	Measured particle size by SEM (nm) <sup>b</sup>	Lattice values (Å) <sup>c</sup>
blank	pH6	53.1	363±56	a = 4.985(2) c = 5.653(4)
	water	47.2	292±42	a = 4.9823(2) c = 5.6445(2)
Ge 28	pH6	47.2	407±78	a = 4.9853(2) c = 5.6490(3)
	water	47.2	297±39	a = 4.9857(1) c = 5.6493(1)
Native silk	pH6	64.4	785±11	a = 4.9867(2) c = 5.6518(4)
	water	60.7	398±58	a = 4.9886(1) c = 5.6524(2)
Silk-Ge28 10%	pH6	84.9	1306±257	a = 4.9813(5) c = 5.6448(8)
	water	106.2	809±105 1289±264	a = 4.9813(1) c = 5.6441(1)
Silk-Ge28 50%	pH6	85.0	1228±154	a = 4.9819(4) c = 5.6458(6)
	water	106.2	833±199 1421±342	a = 4.9880(2) c = 5.6502(4)

<sup>a</sup> Obtained using the Scherrer equation on plane (101) at 26 2θ. <sup>b</sup> From SEM images averaging 50 particles using Image J. <sup>c</sup> Calculated by Rietveld fitting method. Numbers in parenthesis correspond to standard deviations.



**Fig. 5** FTIR spectra of materials obtained, showing the main vibrations of hexagonal GeO<sub>2</sub> and presence of amide bands attributable to silk protein for material mineralised in the presence of native silk. <sup>10</sup> Insert shows the shift of the Ge-O-Ge vibrational band to higher wavenumber in the presence of additives (dashed line) in comparison with the no additive sample (solid line).

Infrared analysis (Fig. 5) corroborates the formation of the α-quartz phase and low levels of organic content associated with the crystals. The three peaks characteristic of the GeO<sub>2</sub> hexagonal phase were found between 585 and 514 cm<sup>-1</sup> and are attributed to

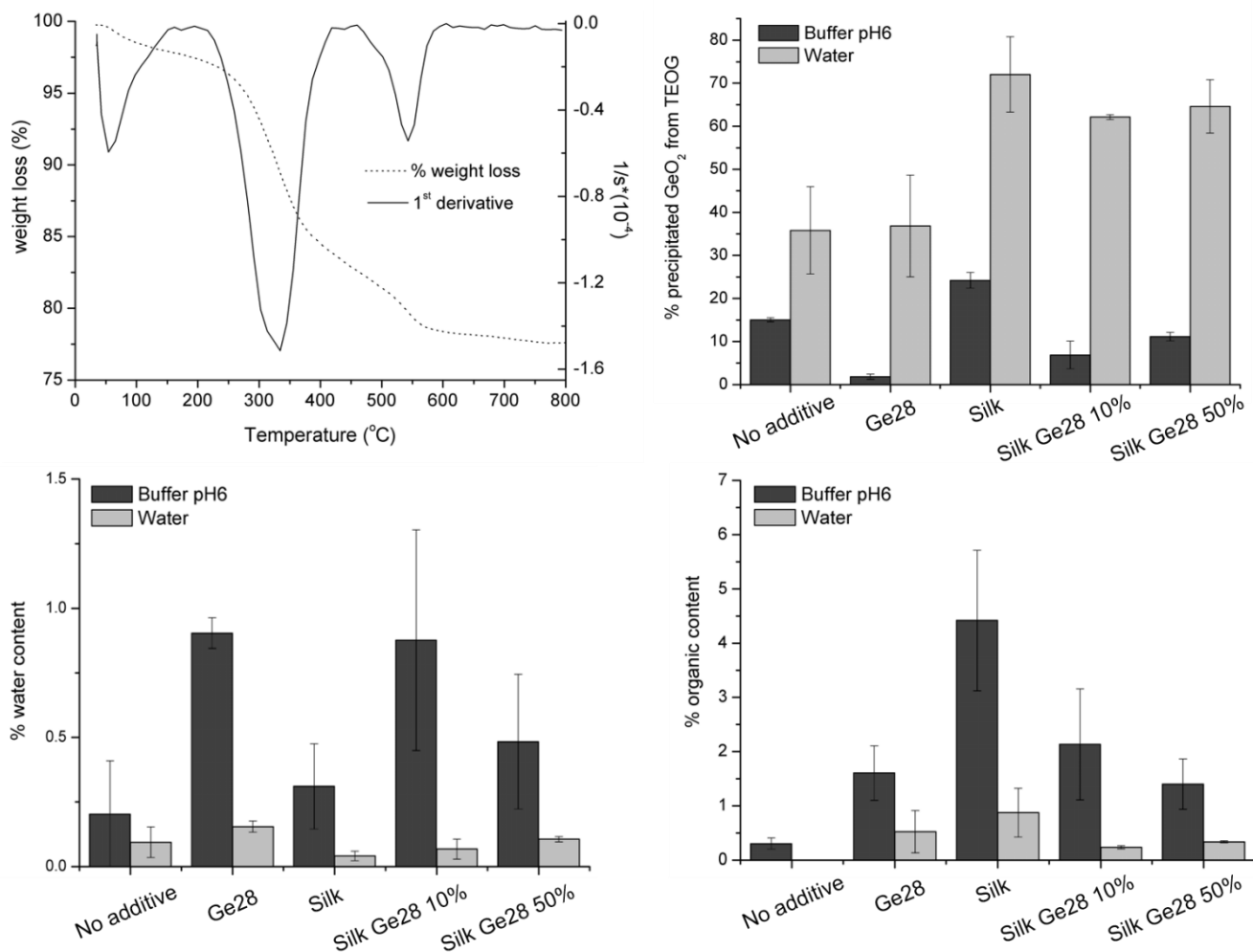


Fig. 6 Sample composition obtained from TGA analysis: (A) example of TGA data of GeO<sub>2</sub>-Silk at pH 6 showing weight loss temperature ranges used for calculation of water and organic content in hybrid materials synthesised. (B) GeO<sub>2</sub> yield; (C) water content and (D) organic content.

the Ge-O-Ge  $\nu_4$  vibrational mode. Peaks arising from the Ge-O-  
 5 Ge antisymmetric stretching mode of hexagonal GeO<sub>2</sub> were found at *ca.* 870 and 960 cm<sup>-1</sup>.<sup>41,42</sup> In the presence of native silk vibrational bands arising from the CO stretch (1600-1700 cm<sup>-1</sup>) and NH bend (1500-1600 cm<sup>-1</sup>) of the amide backbone of the additive<sup>43</sup> were clearly visible. These bands were less intense for  
 10 materials prepared in the presence of the modified silk-Ge28 chimeras or Ge28 peptide (Fig. 5) suggesting that the amount of biomolecule present in the precipitates varied with the identity of the biomolecule used in the synthesis. The insert in Fig.5 shows the shift of the Ge-O-Ge vibrational band from 870 cm<sup>-1</sup> (no  
 15 additive) to higher wavenumber values in the presence of additives in solution suggestive of an interaction between the biomolecules and the germania mineral (Table S.1 ESI†). The biomolecules used are soluble at the concentration range used in this study, therefore the weakly bound organic molecules are  
 20 washed-off during precipitate isolation.

The yield of pure GeO<sub>2</sub> was calculated from the amount of material remaining after thermal treatment (Fig. 6). In general, materials prepared in the buffered system gave 20-60% lower GeO<sub>2</sub> yields than corresponding materials prepared from water.  
 25 In the buffered solutions, all additives (except native silk)

appeared to further inhibit the production of GeO<sub>2</sub> compared to the blank.

#### Effect of additives and buffer

30 Silk proteins clearly promote precipitation when mineralisation was performed in water. The presence of silk and silk chimeras affected the crystallite domain sizes and size of the germania cube-like particles formed. The presence of the silk based proteins was crucial to obtain larger and well defined cube shaped  
 35 germania with larger fundamental crystallite site, while the precise nature of the silk protein used also showed control over particle and crystallite sizes.

Different loadings of peptide Ge28 (HATGTHGLSLSH) coupled to the silk protein in the silk chimeras were used to understand if  
 40 the precipitation promotion and morphologic effect were due to the silk chain, or to the presence and quantity of the peptide in the chimeras. Native silk is rich in acidic residues whereas the silk chimeras have a higher content of basic amino acids contributed from the peptide (Table 1). It was interesting to note however,  
 45 that variation in chimera peptide content did not affect the mineral crystal growth. We expect that the addition of peptides to the chemical chimeras changed the conformation and solution

properties of the chimeric protein compared to native silk, possibly affecting mineralisation and thus leading to larger particles/crystallites being formed.

Peptide Ge28 was previously identified as a germania binder by a combinatorial method.<sup>22</sup> Peptides identified by biopanning, rich in hydroxyl and imidazole containing amino acids have been previously shown to promote the formation of interconnected amorphous germania nanoparticles from a 0.135M TMOG solution in methanol<sup>22</sup> though the fate of the peptide (inclusion or not in the precipitated solid) was not reported. At our precursor concentration (TEOG, 0.2M), precipitation in the presence of Ge28 (HATGTHGLSLSH) gives hexagonal GeO<sub>2</sub>. However, the precipitation activity is very low in spite of the presence of imidazole groups (His) and hydroxyl groups (T) in the peptide. The formation of hexagonal cube-like crystalline particles in the mineralising system used is expected at the precursor concentration used (0.2 M) in agreement with previous results.<sup>20</sup> The presence of basic amino acids, in particular of Histidine, in peptides has been proposed to lead to GeO<sub>2</sub> surface recognition and enhanced precipitation activity.<sup>22</sup> The inactivity of Ge28 in our study clearly suggests that the presence of imidazole or hydroxyl groups in a molecule is not sufficient to induce germania mineralisation under the conditions used. Although the hydroxyl and histidine functionalities may have a clear role in surface recognition, catalytic activity may depend on other factors. As previously suggested for silica condensation reactions, in addition to the amino acid active groups, the conformation of the additive,<sup>5</sup> the distribution of hydrophobic/hydrophilic residues,<sup>7</sup> the presence and position of specific amino acids and motifs in the peptide chain<sup>6</sup> may all play a key role in mineral formation.

Previous studies have shown that crystallinity of the GeO<sub>2</sub> obtained by biomimetic approaches varies depending on the bio-additive. A reaction system containing an amphiphilic peptide resulted in amorphous germania,<sup>2</sup> whereas basic amino acids such as lysine lead to crystalline GeO<sub>2</sub>.<sup>20</sup> In these cases, the formation of crystals was also found to be dependent on GeO<sub>2</sub> concentration.<sup>20</sup> Generally, an interaction between the anionic germanate species arising from hydrolysis of the precursor and the cationic groups present in the additives has been proposed to be responsible for recognition and mineralisation.<sup>21</sup> However the variety of synthetic conditions used as well as non-comprehensive materials characterisation has so far hindered a clear understanding of the specific role of bio-additives in determining crucial properties of germania including intrinsic crystallinity and particle size.

The composition of the mineralisation system has a remarkable effect upon the size of cubic particles obtained. The presence of pH 6 buffer in the synthesis medium dramatically affected the yield, the level of incorporation of organic material and the extent of hydration of the isolated materials. For syntheses conducted in water, addition of silk and silk chimera additives to the reaction medium increased the yield of germania formed, the samples contained lower levels of organic matter and were less-hydrated. The small yields obtained in the presence of the buffer appear to correlate with the size of cubes formed, with reactions performed in the presence of Ge28 showing the lowest yield and smallest particle size as compared to materials formed in the presence of

the silk-based proteins, with highest yields and larger cubes being formed. The formation of precipitable germania was also inhibited in the control sample (no additive) when the chosen buffer was introduced into the reaction system.

The use of silk proteins as modifiers results in the organised growth of the larger GeO<sub>2</sub> nanocubes and crystals. The facilitated growth can be ascribed to the presence of the silk proteins, in particular the chimeric proteins on the minerals' surface, by means of weak interactions during mineralisation. The presence of silk proteins may effectively channel germanate ions on the growing surface, allowing for better organised growth. In the presence of buffer, competition from buffer ions leads to lower growth and reduction in Germania yield.

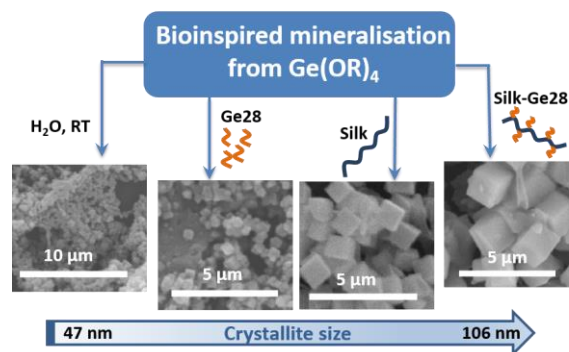


Fig. 7 GeO<sub>2</sub> nanocubes of controlled particle and crystallite size obtained by bioinspired mineralisation using different bioadditives.

## Conclusions

Hybrid materials composed of well-defined cube-shaped polycrystalline hexagonal germania with low organic content (up to 5%), with controllable particle and crystallite size were prepared using silk based biomolecules (Fig. 7). Mineralisation in two condensing systems at circumneutral pH was evaluated: buffered pH 6 (citric acid/bis-tris propane) and water. The effect of additives and mineralisation environment were studied, revealing a drastic impact on mineralisation yield and morphology of the precipitates obtained. During mineralisation in water, native silk and silk chimeric proteins had a double effect: they dictated the GeO<sub>2</sub> particle and crystallite size as well as acting as a catalyst promoting the precipitation of hybrid materials. These effects were biomolecule dependent. We suggest that the silk based protein, in particular chimeric proteins, promote GeO<sub>2</sub> crystal growth by an effective ion-channelling mechanism, favouring the organised growth of crystallites. The presence of ions from the buffer was shown to negatively impact mineral formation, manifest by low yields and a decrease in reaction rate. Additionally, the presence of the buffer also modified the composition and properties of the obtained hybrids and increased the amount of retained water and organic material as well as reducing the extent of crystallinity, possibly due to ion competition.

The standardised synthesis conditions and systematic material characterisation used in this study allows us to rationalise how it is possible to control the particle and crystallite size of hexagonal

GeO<sub>2</sub> particles using a bio-mimetic approach at room temperature using silk based proteins and adjustment of the composition of the mineralisation system. This approach can be used to rationally design a bio-catalytic synthesis of germania materials with defined particle and crystallite size with potentially tuneable functional properties. More detailed experimental and computational studies are needed to elucidate silk's interaction with the growing crystal surfaces and corroborate the proposed channelling mechanism.

## 10 Experimental

### Materials

N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC) 98%; N-hydroxysuccinimide (NHS) 98%; germanium tetraethoxide (TEOG) (99.95%); citric acid (99.5%) were obtained from Sigma Aldrich. Bis-tris propane (1,3-Bis(tris(hydroxymethyl)methylamino)propane, 99%) was obtained from Acros Chemicals. Chemicals were used as received. Silk cocoons were obtained from Forest Fibers, U.K. and regenerated according to the method described below. All solutions were prepared using deionised water (conductivity <1 μS cm<sup>-1</sup>).

### Synthesis of Bio-additives

Peptide Ge28 (HATGTHGLSLSH)<sup>22</sup> was prepared by microwave-assisted solid phase synthesis using the Fmoc chemistry by means of a Discover SPS microwave peptide synthesizer. Peptide purity (>85%, see Fig. S.2, ESI†) and sequence were assessed by RP-HPLC (LC20 chromatography enclosure, Dionex, Sunnyvale, CA), and MALDI ToF mass spectrometry (Bruker Daltonics Ultraflex 3 matrix-assisted laser desorption ionization), respectively, before use in mineralisation experiments.

Native silk was obtained from regeneration of Bombyx silk from silk cocoon including both heavy and light chain as previously reported. Chimeric proteins silk-Ge28 10% and silk-Ge28 50% were prepared by a two steps chemical method as described elsewhere involving peptide coupling to silk active sites by diazotization and EDC/NHS coupling.<sup>33</sup> Silk-peptide chimeric proteins were purified using disposable PD10 desalting columns (Sephadex G-25 Medium, GE Healthcare), lyophilised and stored at -20°C. The coupling process was followed by UV-vis spectrophotometry (Varian Cary 50 UV-vis) (Fig.S3, ESI†).

### GeO<sub>2</sub> mineralisation

Germania mineralisation from 0.2 M TEOG solution was carried out in the presence of the different biomolecules as additives. In a typical experiment, 0.044 ml of TEOG was added to 0.956 ml of a 1 mg/ml solution of additive in distilled water or in buffered solution. The buffer was citric acid and bis tris propane at pH 6. The mixture was stirred for 48 hours and the precipitate isolated by centrifugation (10,000 rpm, 10 min), rinsed three times with distilled water and lyophilized prior to characterization. Solution pH was monitored during condensation in the water system using an InLab Micro combination glass pH meter (METTLER Toledo). For reactions performed in the presence of buffer, pH was measured at the beginning and at the end of the reaction.

### Materials characterisation

Particle morphology and composition were assessed by scanning electron microscopy (SEM) using a JEOL JSM-840A microscope operated at 15 kV, equipped with an energy-dispersive X-ray analysis (EDX) system with light element detection (Oxford Inca). Samples were attached to a carbon adhesive tape on an aluminium stub and gold coated for imaging (Edwards, Sputter coater S150B). Particle size was measured from the SEM images using Image J software.

Non-mineral content was measured by Thermogravimetric analysis (TGA) using a TGA 2050 analyzer (TA instruments). Samples (0.5-1.5 mg) were heated in alumina ceramic crucibles from 30°C to 800°C at 10°C/min under flowing air. Weight loss below 200 °C was used to calculate the Water content, while the organic content was calculated from the weight loss between 200 and 700 °C. The % organic content and % water content are expressed as % of the precipitated material. The inorganic content was then calculated by difference and attributed to pure GeO<sub>2</sub>. The germania precipitation yield (%) was calculated from the amount of pure GeO<sub>2</sub> in relation to the TEOG added (as GeO<sub>2</sub>) and expressed as the average of TGA analysis of samples from three separate condensation experiments.

Fourier Transform Infrared Spectroscopy (FTIR) was performed by the KBr method using a Nicolet Magna IR-750, with absorbance measured in the range from 4000 to 440 cm<sup>-1</sup>, averaging 64 scans acquired at 2 cm<sup>-1</sup> resolution.

X-ray diffraction analysis (XRD) was performed on an Oxford Instruments (PANalytical X'Pert PRO) with a CuKα radiation (λ=1.54056Å). Samples were analysed at room temperature in a polyethylene terephthalate (PET) holder and were scanned for 2θ values from 4° to 80° at 45 kV accelerating voltage, scan speed of 0.02 2θ s<sup>-1</sup> and 40 mA filament current. The crystallite domain sizes were determined by applying the Scherrer equation<sup>44</sup> to the 100% relative abundance peak (101), assuming a shape factor (K) of 0.9. Differences in lattice constants of the hybrid materials from those of pure GeO<sub>2</sub> were assessed to estimate the possible intercalation of organic matter in the hybrid material's crystal structure. Lattice constants were calculated by the Rietveld method<sup>45</sup> using Microstructural Analysis Using Diffraction (MAUD) software<sup>46</sup> with a pure P3<sub>1</sub>21 hexagonal GeO<sub>2</sub> pattern (COD ref. 2300365)<sup>47</sup> as reference.

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### Notes and references

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† Electronic Supplementary Information (ESI) available: EDX analysis of materials formed, peptide characterisation, UV vis spectra of silk, diazo-silk and peptide-silk chimera, IR shift of Ge-O-Ge vibration in the presence of biomolecules. See DOI: 10.1039/b000000x/

‡ Footnotes should appear here. These might include comments relevant to but not central to the matter under discussion, limited experimental and spectral data, and crystallographic data.

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