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Identification of peptides that promote the rapid precipitation of germania nanoparticle networks *via* use of a peptide display library†

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Peptides that promote the rapid, room-temperature precipitation of amorphous germania nanoparticle networks from solution have been identified *via* use of a combinatorial peptide display library.

Amorphous silicon dioxide (silica) is routinely produced under physiological conditions by biological organisms through the use of specialized proteins.[1] These organisms exert impressive control over the morphologies of three-dimensional (3D) silica structures. Spectacular examples of protein-mediated 3D assembly of silica nanoparticle structures are provided by diatoms (uni-cellular algae).[2] Each of the 10^4–10^5 extant diatom species assembles a frustule (microshell) with a unique shape and with fine (submicron) features (*e.g.* pores, supporting ribs, channels, protuberances) that are organized into precise, species-specific patterns.[2] The spicules of certain sponges are also composed of amorphous silica. Indeed, glassy spicules produced by the sponge *Euplectella* possess refractive index profiles and waveguiding properties akin to man-made optical fibers.[3] The identification and functional analyses of proteins associated with *in vivo* silica formation, and the use of such proteins to direct the *in vitro* assembly of silica structures with tailored, non-natural shapes (biosculpting), are active areas of research.[1,4]

Silica is the predominant constituent of a wide variety of commercial glasses, ranging from common window glasses to advanced glasses used in high-bandwidth optical fibers. In order to achieve an expanded range of properties, silica-based glasses can be alloyed with other glass-forming oxides, such as germanium oxide (germania). Silica–germania glasses possess higher refractive indices, and lower viscosities, than pure silica.[5] Furthermore, germania-based glasses possess reduced phonon energies and enhanced transmission at infrared wavelengths relative to silica-rich glasses.[6] As a result, germania-based glasses are receiving increased attention for use in integrated optical lasers, sensors, display devices, and amplifiers.[6]

While natural silica-forming proteins have been identified by several authors, germania-forming proteins have yet to be found in nature. The objective of this paper is to identify peptides that promote germania precipitation, in order to allow for future *in vitro* biosculpting of germania-based assemblies with tailored shapes. A commercially available combinatorial peptide display library was used to identify peptides that exhibit strong binding to germania.[7,8] After several rounds of selective panning, 21 peptide-displaying phage clones with an enhanced affinity for germania were isolated. Preliminary experiments using a PCR method previously described[8e] failed to yield any phage clones that remain attached to the germania particles after acid elution. This indicated that the acid elution was effective in releasing all of the germania-binding peptide displaying phages. The amino acid sequences of the displayed peptides were determined using DNA sequencing. The 3 most dominant peptides identified from the clones, labeled Ge2, Ge8, and Ge34 in Table 1, were chosen for further evaluation.

† Electronic Supplementary Information (ESI) is available describing the biopanning, germania precipitation assay, and molybdenum blue assay procedures. See http://www.rsc.org/suppdata/cc/b4/b402480j/

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In order to assess the ability of these germania-binding peptides to promote germania precipitation, the peptides were introduced (1 mg^{-ml}) into a germanium alkoxide-bearing solution (0.135 M tetramethoxygermanium dissolved in methanol) at room temperature. Precipitation occurred rapidly upon introduction of either the Ge8 or Ge34 peptide into the alkoxide solution. In contrast, germania precipitation induced by the Ge2 peptide was difficult to detect by visual observation. Control experiments conducted with non-germania-binding peptides AG5[8a] and AG-P28,[8e] or in the absence of the germania-binding peptides, failed to yield germania precipitates from the alkoxide solution. The extent of germania precipitation (*i.e.* the germania precipitating activity) was quantified by adapting the β-silicomolybdate colorimetric assay.[9] As shown in Fig. 1, the Ge8 and Ge34 peptides exhibited relatively high germania-precipitating activities, whereas the Ge2 peptide exhibited much lower activity. The control peptides, AG5 and AG-P28, showed no germania precipitating activity.

The amino acids within peptides can provide molecular recognition motifs for strong binding to specific inorganic surfaces. The molecular characteristics that result in strong binding of a particular peptide to a specific inorganic surface may also enable that peptide to enhance the precipitation of the inorganic solid from a solution. The germania-binding peptides that were particularly effective in promoting germania precipitation from an alkoxide solution, Ge8 and Ge34, possessed hydroxyl- and imidazole-containing amino acid residues. The Ge8 peptide possessed a more basic isoelectric point (pI), and a higher germania precipitating activity, than the Ge34 peptide. The germania-binding peptide with a low germania precipitating activity, Ge2, lacked histidine residues and possessed a more acidic pI. The failure of the control peptides AG5 and AG-

Table 1 Amino acid sequences and calculated isoelectric points (pI) of peptides used in this study

Peptide	Amino acid sequence	pI[a]
Ge2	TSLYTDRPSTPL	5.50
Ge8	SLKMPHWPHLLP	8.51
Ge34	TGHQSPGAYAAH	6.61
AG5	SLATQPPRTPPV	9.47
AG-P28	SPLLYATTSNQS	5.24

[a] pI calculated using pI/mass program at www.expasy.ch.

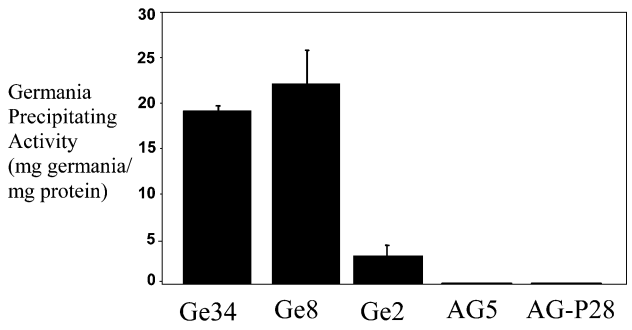

Fig. 1 Germania precipitating activity of germania-binding and control peptides (milligrams of germania formed per milligram of peptide).

P28 to produce germania indicated that precipitation in the presence of the germania-binding peptides was not a simple pH mediated hydrolysis but was instead specific to those sequences isolated by the panning process. It is worth noting that similar molecular characteristics (hydroxyl- and imidazole-containing amino acid residues, basic pI) have been found in peptides that exhibited a high silica precipitating activity,[8] although these silica-precipitating peptides were not among the 21 germania-binding peptides isolated in the present work. Further research is underway to identify the unique molecular characteristics of germania-binding peptides that promote germania precipitation. The influence of precipitation conditions (temperature, alkoxide concentration, solution pH, *etc.*) on the germania morphology is also under investigation.

The germania precipitates generated in the presence of either the Ge8 peptide or the Ge34 peptide were characterized by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). SEM micrographs (Figs. 2(a) and (b)) revealed that the precipitation products consisted of porous, inter-connected networks of agglomerates of fine particles. Energy dispersive X-ray (EDX) analyses revealed that the precipitates were enriched in germanium and oxygen (Fig. 2(e); the minor Au and Pd peaks were a result of the coating applied to the particles to avoid surface charge buildup in the SEM, and the minor Si peak was generated by the silicon substrate on which the precipitates were placed). High resolution TEM images (Figs. 2(c) and (d)) confirmed the open network structures and revealed that the fundamental germania particles possessed diameters of the order of 50–100 nm. TEM analyses also revealed that the particle agglomerates contained regions of low density (relatively bright regions in Figs. 2(c) and (d)), which consisted of either entrapped pores or residual organic material. Electron diffraction (ED) analyses obtained at numerous locations within the Ge8- and Ge34-induced precipitation products indicated that the germania was amorphous. The morphologies of germania precipitates formed in the presence of the Ge8 and Ge34 peptides were similar.

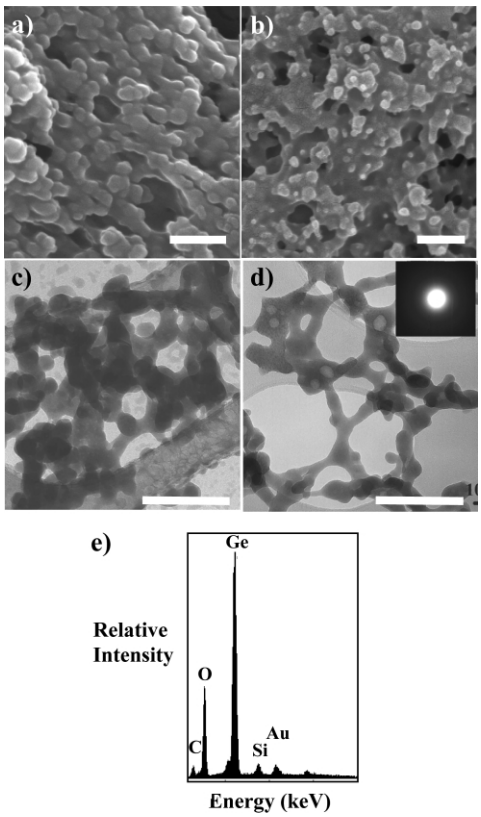

Fig. 2 Germania nanoparticle networks precipitated from an alkoxide solution in the presence of germania-binding peptides. (a), (b) SEM images of germania formed in the presence of Ge8 and Ge34 peptides, respectively; (c), (d) TEM images of germania formed in the presence of Ge8 and Ge34 peptides, respectively; (inset) ED pattern of Ge34 precipitate; (e) EDX analysis of precipitate obtained using the Ge34 peptide. Scale bar, 250 nm.

The combinatorial peptide display technique (so-called biopanning) can be a rapid and effective means of identifying peptides that will bind selectively to, and promote the rapid precipitation of, technologically important solids for a host of electronic, biomedical, optical, chemical, sensor, and catalytic applications.[7,8] In this study, we have used a phage display peptide library to identify germania-binding peptides that promote the rapid, room-temperature precipitation of amorphous germania nanoparticle networks from an alkoxide solution. It is likely that the chemistry, and resulting optical properties, of the germania-bearing precipitates can be adjusted by doping of the alkoxide precursor solution and/or by using combinations of peptides that promote the co-precipitation of germania with other oxides (*e.g.* germania–silica compositions for optical waveguides). The ability of such peptides to promote the *rapid, room-temperature precipitation of tailored oxide compositions*, coupled with the relative ease of peptide patterning on various surfaces, enables exciting new opportunities for the integration of functional oxides with low-temperature or reactive materials (*e.g.* polymer-, bio-organic-, or silicon-based devices).

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