Metallopolymer–Peptide Hybrid Materials: Synthesis and Self-Assembly of Functional, Polyferrocenylsilane–Tetrapeptide Conjugates

Siree Tangbunsuk, [a] George R. Whittell,[a] Maxim G. Ryadnov,[a, b] Guido W. M. Vandermeulen,[c] Derek N. Woolfson,*[a, d] and Ian Manners*[a]

Abstract: Conjugates of poly(ferrocenylmethylsilane) (PFDMs) with Ac-(GA)3EG-OH, Ac-AcOH, Ac-Gl-OH and Ac-VC-OH have been prepared by reaction of the tetrapeptide units with the amino-terminated metallopolymer. The number average degree of polymerisation (DPn) of the PFDMs was approximately 20 and comparable materials with shorter (DPn≈10) and/or amorphous chains have been prepared by the same procedure. Poly(ferrocenylmethylsilane) (PFEMS) was employed for the latter purpose. All conjugates were characterised by GPC, MALDI-TOF MS, NMR and IR spectroscopy. With the exception of Ac-VF, PFDMs20, all materials exhibited some anti-parallel β-sheet structure in the solid state. The self-assembly of the conjugates was studied in toluene by DLS. The vast majority of the materials, irrespective of peptide sequence or chain crystallinity, afforded fibres consisting of a peptidic core surrounded by a PFS corona. These fibres were found in the form of cross-linked networks by TEM and AFM. The accessibility of the chemically reducing PFS corona has been demonstrated by the localised formation of silver nanoparticles on the surface of the fibres.

Introduction

The combination of peptide and synthetic polymers in bioconjugates has afforded hybrid materials that benefit from the presence of each component.[1–6] For instance, the second conjugates have afforded hybrid materials that benefit from the amino-terminated metallopolymer. The number average degree of polymerisation (DPn) of the PFDMs was approximately 20 and comparable materials with shorter (DPn≈10) and/or amorphous chains have been prepared by the same procedure. Poly(ferrocenylmethylsilane) (PFEMS) was employed for the latter purpose. All conjugates were characterised by GPC, MALDI-TOF MS, NMR and IR spectroscopy. With the exception of Ac-VF, PFDMs20, all materials exhibited some anti-parallel β-sheet structure in the solid state. The self-assembly of the conjugates was studied in toluene by DLS. The vast majority of the materials, irrespective of peptide sequence or chain crystallinity, afforded fibres consisting of a peptidic core surrounded by a PFS corona. These fibres were found in the form of cross-linked networks by TEM and AFM. The accessibility of the chemically reducing PFS corona has been demonstrated by the localised formation of silver nanoparticles on the surface of the fibres.

Keywords: nanoparticles • peptides • polymers • self-assembly • supramolecular chemistry

[a] Dr. S. Tangbunsuk, Dr. G. R. Whittell, Dr. M. G. Ryadnov, Prof. D. N. Woolfson, Prof. I. Manners
School of Chemistry, University of Bristol
Cantock’s Close, Bristol BS8 1TS (UK)
Fax: (+44) 117-925-1295
Fax: (+44) 117-929-0509
E-mail: D.N.Woolfson@bristol.ac.uk
ian.manners@bristol.ac.uk

[b] Dr. M. G. Ryadnov
National Physical Laboratory, Hampton Road
Teddington, Middlesex, TW11 0LW (UK)

[c] Dr. G. W. M. Vandermeulen
BASF, Carl-Bosch-Strasse 38
67056 Ludwigshafen (Germany)

[d] Prof. D. N. Woolfson
Department of Biochemistry, University of Bristol
University Walk, Bristol BS8 1TD (UK)

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.201102223.

Complementing the solid-state studies performed by Sogah to be realised.[13–16] Nonetheless, the majority of work has been focused on the incorporation of β-sheet-forming amino acid sequences,[17,18] as these are key components of many structurally interesting natural products, such as amyloid fibres, natural silks and elastin.

It was a desire to understand the mechanism for the aggregation of the amyloid precursor protein that led Lynn and co-workers to couple an oligopeptide with a polyethylene glycol (PEG).[19–21] They found that addition of PEG to the C terminus allowed for the formation of β-sheets that was common to the native protein, but inhibited further aggregation to afford soluble fibrils. By replacing the amorphous segments of the proteins comprising Bombyx mori and Nephila clavipes silks with PEG, Sogah and co-workers have obtained materials that retained the anti-parallel β-sheet structure characteristic of the natural product, along with the associated desirable mechanical properties.[22–24] Shao and co-workers have also prepared silk-inspired copolymers by conjugating A5 (A=alanine) with isoprene oligomers.[25] The general ability of PEG to prevent the lateral aggregation of β-sheet-forming sequences has been prevented by conjugation with another peptide, namely, Ac-QKFOFQEQQ-NH2 (Ac—acetyl, Q—glutamine, K—lysine, F—phenylalanine, E—glutamic acid) for which the conjugate formed soluble fibrils, whereas the parent peptide afforded a hydrogel in solution.[26] Utilising protein engineering and well-defined PEG oligomers, the group of van Hest prepared the monodisperse conjugates, PEG-[(AG)3EG]n-PEG (n = 10 and 20; G = glycine), which also contained the silk-inspired alanilgycine repeating unit.[27] Complementing the solid-state studies performed by Sogah...
and co-workers, the authors found that these materials self-assemble from solution to afford well-defined fibrils, the widths of which are related to the length of the β-sheet-forming block. The same group also prepared a conjugate containing the oligopeptide sequence VPGVG (V = valine, P = proline), common to tropoelastin, located in the side-chain structure.\[9]\] Although combined with a polymethacrylate instead of PEG, this material still displayed the lower critical solution temperature (LCST) common to the peptide. Castelletto and Hamley have also obtained fibrillar structures based on β-sheets by using F\_4 as the aggregator domain.\[28]\] This work has been contrasted with V\_4-containing conjugates and the effect of PEG molecular weight has also been investigated.\[39]\] Recently, Tam and co-workers have prepared conjugates of poly(acrylic acid) with poly(l-valine) and demonstrated that the β-sheet structure also dominates in solution for these materials.\[30–32]\]

In addition to having linear and side-chain conjugates, structurally intermediate materials have been realised that contain reverse-turn mimics. Sogah and co-workers employed a phenoxathiin derivative in this role and demonstrated the ability of this rigid spacer to template intramolecular hydrogen bonding.\[22,23]\] More recently, Börner and co-workers have employed a carbazole unit to pre-organise two (TV)\_2 (T = threonine) units for intermolecular interactions.\[33]\] It is noteworthy that this material exhibited a superior tendency to aggregate than the un-templated conjugate containing (TV)\_4. In further work with aggregator domains based on (TV)\_4, the same group have devised functional systems in which the self-assembly of the conjugate can effectively be “switched-on” by chemical changes to a peptidic precursor.\[34,35]\]

The use of PEG as the synthetic component of the conjugate has enabled the self-assembly of these materials to be studied in aqueous solution. Utilising poly(n-butylacrylate), however, Börner and co-workers attempted similar processes in organic media.\[36]\] The use of non-polar solvents eliminates any contribution to self-assembly from the hydrophobic effect, allowing hydrogen bonding alone to dictate the morphology. These studies afforded wound-tape structures containing anti-parallel β-sheet cores. Since this report, other groups have also achieved the self-assembly of peptide–polymer conjugates in organic solvents by utilising hydrophobic chains.\[37,38,41]\] The use of functional polymers for the stabilisation of β-sheet structures, however, has been limited. Frauenrath and co-workers employed an elegant approach in which diacetylenes were incorporated into the conjugate, which could be converted into functional polyacetylenes post self-assembly.\[37,42,43]\] Preformed, semiconducting oligoithiophenes have also been self-assembled as part of a peptide conjugate.\[44]\] Herein, as an extension to our previous communication, we report the synthesis of a range of tetrapeptide conjugates with polyferrocenylsilanes (PFSs), a class of functional metallopolymers, constituting the functional synthetic component. The self-assembly of these materials in organic media was investigated and the effect of this process on the functionality of the PFS was evaluated.

Results and Discussion

To investigate the effect of amino acid sequence, as well as synthetic-polymer molecular weight and crystallinity on the observed solution-phase self-assembly of the conjugates, we embarked on the synthesis of ten materials. Inspired by the crystalline region of B. mori silk, we decided to couple Ac-(GA)\_2-OH to poly(ferrocenylmethylsilane) (PFDMS). In all cases the tetrapeptides were acylated at the N terminus (denoted by Ac-), and were coupled to the polymer at the C terminus, which existed as the free acid (denoted by -OH). The role of the alanlyglycine repeating units in self-assembly was then judged by comparison with analogous materials containing Ac-G\_4-OH and Ac-A\_4-OH. Finally, Ac-V\_4-OH was studied because of the strong propensity for this amino acid to form β-structures in natural proteins.\[45]\] PFDMS is a semicrystalline polymer and thus, to probe the effect of crystallinity on the self-assembly process, we also prepared an analogue containing the amorphous poly(ferrocenylethylsilane) (PFEMS). The synthetic polymer is considered to play a key role in preventing the lateral aggregation of β-sheets and we have, therefore, also chosen to vary the number of repeat units constituting this block (degree of polymerisation, DP\_n \approx 10 and 20). Finally, we have examined the effect of the specific amino acid sequences upon self-assembly. In non-polar solvents, which are thermodynamically good for the PFS block, hydrogen bonding is expected to be the dominant force governing self-assembly. Although the number of H-bond donors and acceptors in all native tetrapeptides will be the same, the hydrophaphy of the side chain\[46]\] relative to PFS may be expected to play a role in determining the morphology.

Synthesis and characterisation of Ac-X\_4-PFS conjugates: PFDMS and PFEMS were both prepared by living anionic ring-opening polymerisation of the appropriately substituted disila[1]ferrocenophane by using n-butyllithium as the initiator (Scheme 1). The reactions were quenched with 1-(3-bromo-2,2,5,5-tetramethyl-1-aza-2,5-disilacyclopentane and, after purification, amino-terminated PFSs were obtained as light orange solids.\[41]\] All four materials were characterised by 1H NMR spectroscopy, gel permeation chromatography (GPC) and matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS). The results of these various analyses (Table 1) were in close agreement with the target DP\_n values of 10 and 20.

The tetrapeptide sequences were prepared by solid-phase peptide synthesis utilising standard 9-fluorenlymethoxycarbonyl (Fmoc) chemistry.\[47]\] These were characterised by 1H NMR spectroscopy and electrospray ionisation mass spectrometry (ESI MS) before being coupled in ten-fold excess with the amino-terminated PFSs by using PyBOP and DIPEA (Scheme 1). The Ac-X\_4-PFS conjugates were ultimately obtained as orange powders in high yield (e.g., 72% for Ac-A\_4-PFDMS\_20).
The conjugates were soluble in good solvents for PFS, such as toluene, tetrahydrofuran (THF) and chlorocarbons, which allowed for solution-phase characterisation by GPC and $^1$H NMR spectroscopy (Table 2). The chromatograms from the former were monomodal but indicated number-averaged molecular weights that were consistently lower than those determined by MALDI-TOF MS. The $^1$H NMR spectra for the proposed conjugates exhibited resonances assignable to the tetrapeptides, and thus afforded evidence that the coupling reaction was indeed successful. Definitive support for conjugate formation was provided by MALDI-TOF MS, which exhibited peaks that coincided with the values calculated from the molecular formulae (Table 2). It is noteworthy that no signals corresponding to the amino-terminated polymer could be detected by this technique. The MALDI-TOF MS spectra of two representative Ac-X$_r$-PFS conjugates are displayed in Figure 1. An inspection of the peak-to-peak mass differences reveals a value of 242 g mol$^{-1}$ for Ac-A$_4$-PFDMS$_9$ and 256 g mol$^{-1}$ for Ac-V$_4$-PFEMS$_9$. These masses are consistent with the composition of the repeat units of the respective PFS chains.

Physical properties of the Ac-X$_r$-PFS conjugates: The thermal properties of the PFS-tetrapeptide conjugates were investigated by thermal gravimetric analysis (TGA) and differential scanning calorimetry (DSC). By way of comparison, the amino-terminated precursor polymers were also studied and these results are presented, along with those of representative conjugates, in Table 3. The amino-terminated polymers (PFDMS$_{20}$-NH$_2$ and PFEMS$_{18}$-NH$_2$) started to decompose under a nitrogen atmosphere at approximately 429 and 403°C, respectively. Decomposition was essentially complete by 600°C, affording an involatile material with a yield of approximately 36%.
This behaviour is similar to that observed for high molecular
weight linear PFDMS,[43] with the exception that two distinct mass-loss processes were reported.

The TGA thermograms for the conjugates showed two processes (Table 3), with that at lower temperature always corresponding to the smaller percentage mass decrease. The magnitude of this change is in close agreement with that predicted theoretically for complete loss of the tetrapeptide segment (ca. 6 and 11% mass reductions for the high and low molecular-weight materials, respectively). The observed discrepancy most probably arises from limited decomposition/polymerisation of the metallopolymer chain. If the second mass-loss process is taken to correspond to formation of the α-Fe-containing ceramic generally afforded upon pyrolysis of linear PFDMS under nitrogen, then the percentage mass loss is larger than expected, especially for the high molecular-weight materials. The presence of the tetrapeptide would, therefore, appear to aid polymerisation and thus reduce the ceramic yield from the PFS block.

DSC thermograms were acquired on all materials as three cyclic scans between −60 and 300 °C. The first heating ramp was used to anneal the sample and thereby erase all thermal history. The thermograms of the PFEMS-containing materials exhibited no first-order transitions that could be assigned to melting or crystallisation processes involving the semicrystalline metallopolymer block. This observation presumably arises due to the crystallisation kinetics being slow relative to the time scale of the experiment. As a result, the only feature observed was a second-order process (19–25 °C), in accordance with predictions for complexes of the metallopolymer crystallinity and block length seemed to have no effect on the propensity of the material to exhibit an anti-parallel β-sheet structure. In addition to absorptions corresponding to an anti-parallel β-sheet (1685, 1632 and 1556 cm−1), the FT-IR spectrum of Ac-GA2-PFEMS18 exhibited characteristic absorptions of an anti-parallel β-sheet prior to thermal annealing (Table 4). This behaviour has since proven to be the norm with the majority of materials containing domains with an anti-parallel β-sheet structure upon simple precipitation. In the cases of the (GA)2 and A4 peptide segments, metallopolymer crystallinity and block length seemed to have no effect on the propensity of the material to exhibit the anti-parallel β-sheet structure. If the anti-parallel β-sheet structure. In addition to absorptions corresponding to an anti-parallel β-sheet (1685, 1632 and 1556 cm−1), the FT-IR spectrum of Ac-GA2-PFEMS18 exhibited characteristic absorptions of an anti-parallel β-sheet prior to thermal annealing (Table 4). This behaviour has since proven to be the norm with the majority of materials containing domains with an anti-parallel β-sheet structure upon simple precipitation.

**Table 3. Thermal properties of the amino-terminated PFS homopolymers and representative Ac-A4-PFS conjugates.** Scans were performed at a rate of 10°C min−1 for both TGA and DSC.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Tg [°C]</th>
<th>Ta [°C] ([%])b[a]</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFEMS18-NH2</td>
<td>19</td>
<td>429 (64.52)</td>
</tr>
<tr>
<td>PFEMS20-NH2</td>
<td>16</td>
<td>403 (64.29)</td>
</tr>
<tr>
<td>Ac-A4-PFEMS18</td>
<td>25</td>
<td>345 (6.56)/414 (79.29)</td>
</tr>
<tr>
<td>Ac-A4-PFEMS20</td>
<td>23</td>
<td>324 (7.52)/424 (74.16)</td>
</tr>
<tr>
<td>Ac-A4-PFDMS18</td>
<td>20</td>
<td>418 (16.21)/468 (66.18)</td>
</tr>
<tr>
<td>Ac-A4-PFDMS20</td>
<td>18</td>
<td>304 (17.42)/403 (44.32)</td>
</tr>
</tbody>
</table>

[a] Decomposition temperature (Tg) was defined by the point at which 5% of the original mass was lost. [b] Percentage mass lost during each transition is reported in parentheses.

**Table 4. FT-IR data for Ac-X4-PFS conjugates.**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Amide I [cm−1]b[a]</th>
<th>Amide II [cm−1]b[b]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Random-coil[b]</td>
<td>1656(s)</td>
<td>1535(s)</td>
</tr>
<tr>
<td>Anti-parallel β-sheet[b]</td>
<td>1632(s), 1685(w)</td>
<td>1530(s)</td>
</tr>
<tr>
<td>Ac-(GA)2-PFEMS18</td>
<td>1650(s)</td>
<td>–</td>
</tr>
<tr>
<td>Ac-(GA)2-PFDMS18</td>
<td>1695(w)[c]</td>
<td>1620(s)[d]</td>
</tr>
<tr>
<td>Ac-(GA)2-PFDMS20</td>
<td>1624 (s), 1694(w)</td>
<td>1535</td>
</tr>
<tr>
<td>Ac-(GA)2-PFEMS20</td>
<td>1624(s), 1694(w)</td>
<td>1539</td>
</tr>
<tr>
<td>Ac-A4-PFEMS18</td>
<td>1626(s), 1694(w)</td>
<td>1540</td>
</tr>
<tr>
<td>Ac-A4-PFDMS18</td>
<td>1626(s), 1693(w)</td>
<td>1540</td>
</tr>
<tr>
<td>Ac-A4-PFDMS20</td>
<td>1627(s), 1692(w)</td>
<td>1541</td>
</tr>
<tr>
<td>Ac-A4-PFEMS18</td>
<td>1625(s), 1693(w)</td>
<td>1539</td>
</tr>
<tr>
<td>Ac-GA2-PFDMS18</td>
<td>1632(s), 1640(s), 1685(w), 1746</td>
<td>1556</td>
</tr>
<tr>
<td>Ac-GA2-PFEMS18</td>
<td>1631(s), 1683(w)</td>
<td>1552</td>
</tr>
<tr>
<td>Ac-V4-PFEMS18</td>
<td>1630(s), 1694(w)</td>
<td>1545</td>
</tr>
<tr>
<td>Ac-V4-PFEMS20</td>
<td>1630(s), 1690(w)</td>
<td>1543</td>
</tr>
</tbody>
</table>

[a] Observed frequencies for amide absorptions of polypeptides in various formations.[51] [b] Letters in parentheses indicate observed intensities (s = strong, m = medium, w = weak). [c] Prior to thermal annealing. [d] After thermal annealing.
interesting behaviour. Those formed with the amorphous metallopolymer (PFEMS) exhibited IR spectra consistent with anti-parallel β-sheet formation, however, that with PFDMS20 gave rise to a single absorption in both amide regions (1630 and 1545 cm\(^{-1}\)), most likely arising from a random-coil conformation of the peptide. These observations are consistent with the relatively long, semi-crystalline metallopolymer block in the latter serving to prevent the crystallisation of the V\(_4\) segment. An anti-parallel β-sheet structure would be expected to be thermodynamically favoured over the parallel arrangement because this minimises the steric interactions between the metallopolymer segments of adjacent conjugates. In the absence of the hydrophobic effect, one would expect further stabilisation of the former as this arrangement maximises the H-bonding interactions. The only materials that do not exhibit an anti-parallel β-sheet structure contain the longer metallopolymer chains. These larger segments would be expected to be more effective at kinetically stabilising the random-coil structure.

**Self-assembly of PFS–tetrapeptide conjugates in solution:**

**TEM studies:** After thermal annealing of Ac-(GA)\(_2\)-PFDMSS\(_{18}\), through three heating/cooling cycles, the material was no longer soluble in toluene. Heating a suspension of this material to 70°C in toluene, however, afforded a stable yellow solution, which increased in viscosity on cooling. This behaviour is consistent with H-bond formation during the annealing process, and their subsequent disruption upon heating in toluene. Analysis of the colloidal solution at room temperature by transmission electron microscopy (TEM; Figure 2) revealed the presence of a fibrous network. Formally exchanging the semi-crystalline metallopolymer for amorphous PFEMS of comparable length (DP\(_n\) = 18) had no obvious effect, with a similar fibrous network being obtained. Furthermore, repeating the procedure with Ac-A\(_2\)-PFDMS\(_{20}\) and Ac-A\(_2\)-PFEMS\(_{18}\) (Figure S2 in the Supporting Information), which contain the aggregator domain common to *Nephila clavipes* silk, afforded the same fibrous networks. Shortening the length of the metallopolymer block, as with Ac-A\(_2\)-PFDMSS\(_9\) and Ac-A\(_2\)-PFEMS\(_9\) (Figure S2 in the Supporting Information), again resulted in formation of the fibrous aggregates, but in this instance no heating was required. Similarly, Ac-V\(_4\)-PFEMS\(_9\) also formed fibres without the need to heat above room temperature (Figure S3 in the Supporting Information). This observation can presumably be ascribed to the reduced steric protection provided by the shorter metallopolymer block, leading to a lower activation barrier to solution-phase aggregation. In contrast, neither Ac-V\(_4\)-PFDMSS\(_{30}\), Ac-G\(_1\)-PFDMS\(_{20}\) nor Ac-V\(_4\)-PFEMS\(_{18}\) exhibited well-defined aggregates upon heating in toluene and cooling to room temperature. This result suggests that Ac-A\(_2\) and Ac-(GA) display a greater tendency to form fibrous structures in dilute toluene solution than Ac-V\(_4\) and Ac-G\(_1\). The propensities of the amino acids to form β-sheet secondary structures are of the order V\(_4\)>A\(_2\)>G\(_1\),\(^{[44]}\) which may account for the observed differences in behaviour between the Ac-A\(_2\) and Ac-G\(_1\) conjugates. Valine, however, is considerably more hydrophobic than both glycine and alanine,\(^{[45]}\) and this is likely to disfavour self-assembly of the Ac-V\(_4\) conjugates in toluene, despite its high propensity to form β-sheets in natural, water-soluble proteins.

**AFM studies:** To confirm that the fibrous structure of the aggregate was not influenced by solvent evaporation within the transmission electron microscope or by the substrate, we also imaged all samples by atomic force microscopy (AFM). Colloidal solutions comparable to those studied by TEM (0.2 mgmL\(^{-1}\) in toluene) were heated appropriately, dip-coated onto silicon wafers and the topology of the material studied in tapping mode. The micrographs revealed the presence of fibrous networks in all instances for which they had been observed by TEM. Representative phase and height images, as well as a sectional profile for Ac-(GA)\(_2\)-PFDMS\(_{18}\), are displayed in Figure 3. The AFM images for Ac-A\(_2\)-PFDMSS\(_9\) and Ac-V\(_4\)-PFEMS\(_{18}\) are shown in Figure S4 in the Supporting Information.

**Effect of temperature:** The observation that colloidal solutions of the conjugates containing the longer metallopolymer blocks required heating to acquire the fibrous structure prompted us to probe the evolution of these mixtures with temperature. To this end, the apparent hydrodynamic radius (R\(_h\)) of a 0.2 mgmL\(^{-1}\) solution of Ac-(GA)\(_2\)-PFDMS\(_{18}\) was measured by dynamic light scattering (DLS) on heating from 25–75°C and similarly on cooling back to the original temperature. The results of this study are displayed in Figure 4. Upon initial dissolution the conjugate exhibited an R\(_h\) of 36 nm, which was significantly larger than expected for individual unimers in solution (see below). Heating had no observable effect until 60°C, at which point R\(_h\) began to increase rapidly, reaching a maximum value of about 150 nm at 70°C. Further heating to 75°C resulted in a slight decrease in size (ca. 130 nm) and this trend continued on cooling with R\(_h\) ultimately tending to a value of 92 nm at 25°C. The assumptions of the Stokes–Einstein model make
a quantitative interpretation of the apparent hydrodynamic sizes difficult, however, there clearly appear to be three characteristic regions: one corresponding to the initial size; another during which the size changes and a third which represents the final fibrous structure.

In an attempt to garner further information regarding the morphology of the aggregates in each region, we recorded TEM micrographs on samples removed from the colloidal solution at 25, 75 and 25 °C, the last being taken after previously heating to 75 °C (Figure 5). In keeping with the relatively large $R_h$ observed upon initial dissolution of the conjugate, the original micrograph at 25 °C is consistent with the presence of large, irregular aggregates in solution. It is also noteworthy that there appear to be a few fibres present, but these can no longer be observed in the micrograph recorded on the sample removed at 75 °C. In this instance, only irregular interconnecting aggregates can be detected. It can be postulated that it is the crystallisation of the oligopeptides in these structures that ultimately affords the observed fibrous network.

In keeping with our initial observations that Ac-A4-PFDMS9 afforded a fibrous structure upon simple dissolution in toluene at room temperature, we observed a temperature invariant $R_h$ of 68 nm by DLS for this conjugate (Figure S5 in the Supporting Information) and confirmed that the fibrous structure was present over the temperature range by TEM (Figure S6 in the Supporting Information). By means of comparison, the conjugates Ac-G4-PFDMS20 and Ac-V4-PFDMS20, which did not form fibrous structures from solution, exhibited $R_h$ values at 25 °C of 84 and 36 nm, respectively. These results suggest that these materials also exist as aggregates in solution.

**Structure of the fibres:** A colloidal solution of the fibrous Ac-(GA)2-PFDMS18 network in toluene was characterised by $^1$H NMR spectroscopy. The spectrum only exhibited signals that could be assigned to the PFDMS block, which suggested that this portion of the macromolecule was solvated and thus formed the corona. This observation was in keeping with our expectation based on the solubility of the material in relatively non-polar solvents. Preliminary measurements by AFM demonstrated that the narrowest fibres varied from 22–50 nm in width and 3–5 nm in height. Analysis of TEM micrographs obtained on fibres formed from the comparable material Ac-(GA)2-PFDMS20 indicate similar thicknesses for the narrowest fibres (ca. 23 nm). This result offers further evidence for the coronal location of the PFS...
block, because in the absence of staining, high electron density is required at the periphery of the fibre to provide the observed contrast. Furthermore, use of AFM to examine the fibres after dip-coating onto a silicon substrate suggested that they consist of bundles of laterally aggregated smaller fibres (Figure 6).

The dimensions of one such fibre when isolated on the substrate were determined to be approximately 7 nm in width and 5.5 nm in height. From the molecular formula of Ac-(GA)₂-PFDMS₂₀, one would predict the tetrapeptide to extend 1.4 nm, with the metallopolymer chain contributing a value between that predicted for a Gaussian coil (ca. 0.5 nm) and the contour length (ca. 3.3 nm). A tape consisting of an anti-parallel β-sheet core of tetrapeptides and a metallopolymer corona would, therefore, be expected to have a width of approximately 2.4–7.0 nm. Börner et al. have studied the self-assembly of poly(n-butylacrylate)₁₈-b-(TV)₅-nPhe-G-OH (nPhe=4-nitrophenylalanine) in organic media and observed the formation of a β-barrel. This structure, consisting of a β-sheet twisted around the long axis of the fibre, was suggested to occur due to the chemical asymmetry of the two faces of the tape. The hydroxyl groups of the threonine residues thus point towards the centre of the cylinder with the isopropyl groups of valine grouped on the outside. The face asymmetry of the tetrapeptides employed in this study is considerably less, although accumulation of residual dipoles on the oligopeptide and sterie repulsion between synthetic-polymer chains may still have been expected to cause the tape to twist. It has been demonstrated, however, that conjugates of similar oligopeptides with polyethylene oxide (PEO) self-assemble in water to afford flat fibres consisting of double β-sheets. This is despite the application of a considerably longer syn-

Figure 5. TEM micrographs of Ac-(GA)₂-PFDMS₂₀ (0.2 mg mL⁻¹ in toluene) recorded at a) 25, b) 75 and c) 25°C (after previously heating to 75°C).

Figure 6. AFM study of Ac-(GA)₂-PFDMS₂₀ (0.2 mg mL⁻¹ colloidal solution in toluene after cooling from 70 to 25°C). a) Phase image annotated with coloured lines and blue arrows to show points at which fibre height and width data, respectively, have been obtained from the corresponding phase image. b) The height profiles along the coloured lines shown in a).
thetic component (DP, ≈73) and in keeping with the increased effective screening of the accumulated charge by the more polar solvent. The van Hest group has also reported the formation of flat fibres from the lateral aggregation of an ABA-type block copolymer that contains [(AG)3EG]20 as the β-sheet element.[25] On the basis of AFM measurements, they postulated that the hydrogen-bonding direction was parallel to the substrate and perpendicular to the long axis of the fibre. Although this is similar to what we have previously suggested for our materials,[19] the dimensions of these fibres, gleaned from more recent experiments, are consistent with coincidence of both the hydrogen bonding and fibril directions (Figure 7). It would be expected that aggregation of the fibres into the observed microscopic network would arise from lateral aggregation of these tapes, occurring only in places in which the kinetic barrier provided by the corona had been overcome. From the work of Lynn and co-workers on the structure of the β-amyloid fibril,[21] such lamination would be expected to have periodicity of approximately 10 nm and this is comparable to the narrowest fibre spacing found by AFM. Furthermore, the side-on orientation of the tapes upon the substrate, which is suggested by these data, is supported by good agreement between the width calculated above and the observed fibre height. The absence of any features in the selective-area electron diffraction pattern of the fibres is also consistent with the proposed orientation of the tetrapeptide core.

**Pattern of silver nanoparticles on Ac-X4-PFS tetrapeptide fibrous networks:** Previous studies have demonstrated that cylindrical micelles formed from the self-assembly of PI-b-PFDMS (PI = polyisoprene) in a selective solvent for PI form cylindrical micelles, which may be made permanent by intra-coronal cross-linking.[54,55] Subsequent dissolution of these objects in a common solvent caused the PFS cores to swell, thus creating space for nanoparticle encapsulation. Utilising both the void created by the swollen core and the redox activity of the PFS chains, we were then able to template the formation of 1D arrays of silver and silver iodide nanoparticles within the core of the micelle.[56] Similar patterns of silver nanoparticles have also been grown on the peptidic cores of self-assembled nanotapes, which were derived from PEO-peptide conjugates.[57] In this case, however, the absence of an in situ reductant enabled the use of visible light to promote the formation of AgI from AgII. The access to fibres with a PFS corona provided by this study (see below) prompted us to investigate the use of the ferrocene/ferroenium redox couple to localise silver nanoparticles in the exterior of the fibres without the need for cross-linking.

Initially, we attempted to synthesise silver nanoparticles on the fibres by adding dropwise a solution of Ag[PF6] in toluene to a colloidal solution of the self-assembled conjugate in toluene. Our reaction was conducted by simply titrating a solution of Ac-(GA)2-PFDMS20 in toluene with a saturated solution of Ag[PF6], also in toluene. The colour of the reaction mixture changed from pale amber to deep yellow and then to dark brown, before a precipitate was finally formed. After centrifugation, we removed the precipitate and obtained a clear, green supernatant liquid. TEM was performed on samples prepared from the supernatant by allowing the solvent to evaporate from a drop on a carbon-coated copper grid. In the micrographs, we observed nanoparticles with diameters ranging from 35 to 110 nm, randomly dispersed on fibres and consistent with the redox-induced preparation of Ag nanoparticles (Figure 8).

We infer that upon addition of Ag[PF6], the AgI ions were reduced to Ag0 by the FeII centres in the PFS corona. This process was accompanied by formation of ferrocenium centres [Eq. (1)].

\[
\text{Fe}^{II}_{\text{PFS}} + \text{Ag}^{I} + \text{Fe}^{III}_{\text{PFS}} + \text{Ag}^{0}
\]

Previous studies have demonstrated in the case of the PI-b-PFDMS cylindrical micelles that pre-oxidation of the PFS with a specific oxidant aids confinement of nanoparticles to the core.[56] We have found, however, that the fibrous structure of the Ac-X4-PFS conjugates when suspended in toluene became unstable after oxidation. Thus, we decided to conduct the pre-oxidation step on a film of the fibrous network before adding Ag[PF6].

The synthesis of the silver nanoparticles on PFS-containing fibres was conducted in two steps, as depicted in Scheme 2. Firstly, thin films of the fibres were prepared by self-assembling Ac-A1-PFDMS20 as a 0.2 mg mL\(^{-1}\) colloidal solution in toluene (see below) and then dip-coating onto a carbon-coated copper grid. The grids were sequentially dipped into a pre-oxidation solution of either tris(4-bromo-phenyl)aminium hexachloroantimonate (Magic Blue) in dichloromethane (0.4 mg mL\(^{-1}\)) or iodine in toluene (0.5 mg mL\(^{-1}\)) and then a solution of the oxidant, Ag[PF6], in toluene (0.5 mg mL\(^{-1}\)).

The reaction products that formed on the copper grid were subsequently analysed by TEM and EDX. The struc-
The features presented in Figure 9 are of Ac-A4-PFDS10 pre-treated with Magic Blue or iodine and then oxidised by Ag[PF6]. TEM demonstrated that pre-oxidation with either Magic Blue or iodine resulted in most silver nanoparticles being located on the fibrous Ac-A4-PFDS10 networks. Furthermore, it would appear that the nature of the pre-oxidant has an effect on both the density of coverage and size of the nanoparticles formed. For instance, the composite material formed after pre-treatment with Magic Blue (Figure 9c) had a higher density (5 particles/10^4 nm^2) of small particles (25 nm), whereas when iodine was employed (Figure 9e) the density was lower (3 particles/10^4 nm^2) and the particles were larger (33 nm). Figure 9d and 9f show the energy-dispersive X-ray spectroscopy (EDX) data of silver nanoparticles on the fibrous networks when pre-oxidised by Magic Blue and iodine, respectively. In addition to the expected silver signals, we also observed signals arising from the presence of chlorine and antimony when the samples were pre-oxidised by the former and iodine when the latter was employed. These data suggest that silver nanoparticle growth on the PFS corona of the fibres is seeded by the precipitation of silver halide and that this process is more efficient in the case of the chloride. A similar mechanism has previously been used to explain the localised formation of similar nanoparticles in the cores of cross-linked PI-b-PFDMs cylindrical micelles.

**Conclusion**

We have synthesised a range of PFS-containing tetrapeptide conjugates and studied the affect of polymer molecular weight (DP, ≈ 10 or 20), crystallinity and specific-peptide sequence on their self-assembly. With the exception of Ac-G4-PFDS20 and Ac-V4-PFEMS18, all of the materials prepared exhibited only anti-parallel β-sheet structures in the solid state. These two conjugates, however, contained some or all of the sequences in a random-coil conformation. Upon dissolution in toluene, all materials with the shorter metallopolymers segments self-assembled to afford fibrous networks with peptide cores and PFS coronae. In the conjugates with Ac-(GA)2 and Ac-A4, lengthening the PFS chain resulted in no observable networks at room temperature, but gave similar results (i.e., fibrous networks). The observed difference was attributed to kinetic stabilisation by the longer chains of what would otherwise be aggregating filaments. The conjugates of either Ac-V4 or Ac-G4 with these larger macro-molecules, however, did not afford comparable networks under any of the conditions attempted. On the basis of detailed studies of the fibres, we have postulated that they are formed by the lateral aggregation of β-sheets. This explanation accounts for the variation in widths and fibre cross-linking. The coronal location of the PFS enables ready access to the reducing iron(II) centres and this was demonstrated in the patterning of Ag0 nanoparticles.
Materials and methods: Alanine, glycine, valine and (benzotriazole-1-yloxy)trispyrrolidinophosphonium hexafluorophosphate (PyBOP) were purchased from Novabiochem. All other commercial chemicals were acquired from Sigma–Aldrich. Amino-terminated poly(ferrocenylidimethylsilane) (PFDMS) and poly(ferrocenylethylmethylsilane) (PFEMS) were both synthesised by using the method published for the former.[41]

All manipulations involved in the preparation of the amino-terminated polyferrocenylsilanes were performed either under dry argon or in vacuo by using standard Schlenk-line and glovebox techniques. Solvents (diethyl ether and hexanes) were purified by using a Grubbs-type solvent system.[43] Tetrahydrofuran (THF) and 1-(3-bromopropyl)-2,2,5,5-tetramethyl-1-aza-2,5-disilacyclopentane were purified by vacuum distillation from the appropriate drying agents (Na/benzophenone and CaH2, respectively) and stored under nitrogen. The tetrapeptides were synthesised by solid-phase peptide synthesis (SPPS) using Fmoc chemistry.[47] The amino acid residues were used as Fmoc-(amino acid)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HBTU) and diisopropylcarbodiimide (DCC; 10% v/v) mixture and the peptide was cleaved by stirring in a trifluoroacetic acid (TFA; 95% v/v), H2O (2.5% v/v) and triisopropylsilane (TIS; 2.5% v/v) mixture and the peptide was precipitated with diethyl ether. All materials were obtained as colourless powders.

Syntheses of Ac-(GA)4-OH, Ac-A4-OH, Ac-G4-OH and Ac-V4-OH: The peptides Ac-(GA)4-OH, Ac-A4-OH, Ac-G4-OH and Ac-V4-OH were synthesised by solid-phase peptide synthesis (SPPS) using Fmoc chemistry.[47] The amino acid residues were used as Fmoc-(amino acid)-OH and couplings were facilitated by use of O-benzotriazole-N,N,N',N'-tetramethyluronium hexafluorophosphate (HBTU) and N,N-diisopropylethylamine (DIPEA). After assembly of the oligopeptide on the Wang resin, the NH2-X-(Wang resin) was stirred in an esterification mixture [acetic anhydride (5% v/v), pyridine (5% v/v), 1,1,2-trifluoroethane (15% v/v)] to obtain Ac-X-(Wang resin). The resin was then cleaved by stirring in a trifluoroacetic acid (TFA: 95% v/v), H2O (2.5% v/v) and triisopropylsilane (TIS): 2.5% v/v mixture and the peptide was precipitated with diethyl ether. All materials were obtained as colourless powders.

Ac-(GA)4-OH: 68 mg, 86% yield; [H NMR (400 MHz, D2O, 19°C, TMS salt): 4.31 (m, 2H; -CH [Ala]), 3.89 (s, 4H; CH2 [Gly]), 2.10 (s, 3H; CH3 [Gly]), 1.20 (s, 3H; CH3) and 0.80 (s, 6H; CH3)].

amino-terminated poly(ferrocenylethylmethylsilanes) were prepared by PFDMS-20-NH2 after consideration of the different PFDMS chain length; MALDI-TOF MS: m/z (%): 2747 (100); GPC: Mw = 5400 g/mol; (Mw/Mn) = 1.30.

Ac-G4-PFEMS(55) % yield: ‘H NMR (400 MHz, CDCl3, 19°C, TMS): 4.20 (brs; Cp), 3.99 (brs; Cp), 2.20 (m, 2H; CH2-NH2), 2.36 (m, 3H; CH3CO-), 1.96 (m; CH2-Si); MALDI-TOF MS: m/z (%): 33% yield; 1H NMR (270 MHz, CDCl3, 19°C): 4.19 (brs; Cp), 3.98 (brs; Cp), 2.34 (m, 2H; CH2-NH2), 2.26 (s, 3H; CH3CO-), 1.97 (t, (J=7 Hz); CH3-CH2-), 0.90 (t, (J=7 Hz); CH3-CH3-); MS (70 eV): m/z (s): 5398 (100); GPC: Mw = 11370 g/mol; (Mw/Mn) = 1.21.

Ac-V2-PFEMS(54) % yield: ‘H NMR spectrum is essentially the same as that for Ac-V2-PFEMS after consideration of the different PFEMS chain length; MALDI-TOF MS: m/z (%): 3115 (100); GPC: Mw = 5930 g/mol; (Mw/Mn) = 1.22.

Acknowledgements

The authors would like to acknowledge the Royal Thai Government Civil Service Commission, European Union, Royal Society (London) and BBSRC for financial support. Kyong Tack Kim for a sample of PFDMNH2 used in the preliminary work and members of the Manners group for helpful suggestions.
