The use of silsesquioxane cages and phage display technology to probe silicone–protein interactions†

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Received (in Montpellier, France) 17th July 2007, Accepted 20th September 2007
First published as an Advance Article on the web 3rd October 2007
DOI: 10.1039/b710984a

A combination of two model approaches was used to explore the nature of silicone–protein interactions. Phage display technology was used to present a combinatorial library of peptides, in this case the phage library PhD.-12, to a series of model silicone surfaces. Solid, molecular cage silsesquioxanes were used as the models for silicone surfaces. The silsesquioxanes were octamethyloctasilsesquioxane (methyl^8-T^8), octaphenyloctasilsesquioxane (phenyl^8-T^8), octahydridooctasilsesquioxane (H^8-T^8) and dodecatrifluoropropyldodecasilsesquioxane (trifluoropropyl^{12}-T^{12}). The first two silsesquioxanes bear simple aliphatic (Me) and aromatic (Ph) pendant groups, and are simple silicone analogues. The second two silsesquioxanes have functionalised pendant groups, H and $CF_3CH_2CH_2$. The panning results, using a wild-type phage as a control, show that the phage library binds to the simple aliphatic and aromatic silsesquioxanes strongly but non-specifically, and largely through the protein coat on the phage. The functionalised silsesquioxanes (H and $CF_3CH_2CH_2$) are bound specifically by the phages. A statistical analysis of the DNA sequences of the strongly binding phages was carried out. The peptides that bind to H^8-T^8 are strongly enriched in proline content at positions 7 and 8, with enhanced histidine at positions 5 and 9, and enhanced threonine at position 11. The proline residues presumably induce a favourable conformation in the peptide for binding to the silsesquioxane surface. The enhanced histidine content is significant. It has been known for many years that imidazole has a particular affinity for electrophilic silanes. The amino acid distribution for trifluoropropyl^{12}-T^{12} binding peptides is markedly different from that of H^8-T^8 silsesquioxane. Proline is again enhanced, but in positions 4, 11 and 12, and there are also very high concentrations of serine in positions 1, 9 and 11, and threonine in positions 1, 2 and 12. The highly polar nature of the CF_3 group is likely to engage in hydrogen bonding with the OH groups of the serine and threonine side chains, accounting for the tight binding to trifluoropropyl^{12}-T^{12} silsesquioxane.

1. Introduction

Silicones are the materials of choice in a great variety of biomedical devices and personal care preparations.[1] Understanding the mode of interaction of silicone materials with biomolecules is therefore important to both improving the efficacy and ensuring the safety of silicone-containing materials.

Silicones present a very high surface hydrophobicity, and they first found commercial use as electrical insulators, reliant upon their water repellency.[2] Silicones continue to be used as water repellents, as well as in many other applications. It is therefore perhaps surprising to find that silicones have a significant affinity for the highly polar biomolecules that are generally found in aqueous environments.[3,4] Brook and co-workers[5] attribute the ability of silicones to bind to biomolecules to "the schizophrenic character of the molecules which contain both polar Si–O linkages and non-polar Me_2Si groups. The very high chain flexibility ... allows the silicone to orient itself at interfaces to favourably interact with hydrophilic and hydrophobic phases".

Proteins are amongst the most important of the biomolecules in the context of their interactions with silicones. Protein binding to silicone surfaces has been known for many years,[6–8] and can have an obvious adverse effect on some silicone-based medical devices. For example, protein deposition on silicone-based contact lenses reduces both their comfort and visual acuity.

The nature and effects of silicone–protein interactions in other contexts are still somewhat controversial, and the science has been briefly reviewed by Brook and co-workers.[5] Brook and co-workers also studied the deposition of an unfunctionalised polydimethylsiloxane (PDMS) and a (triethoxysilyl)-propyl end group-functionalised PDMS onto films of human serum albumin (HSA).[9,10] The interaction was studied by angular-dependant X-ray photoelectron spectroscopy and contact angle measurements. For both silicones, the results

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† Electronic supplementary information (ESI) available: Further experimental data. See DOI: 10.1039/b710984a

suggested a high degree of mixing between the protein and the silicone. The modified silicone was shown to have a greater affinity for HSA, possibly through chemical bonding. Kaufmann and co-workers[11] demonstrated that silicone oil (largely PDMS) can induce aggregation in four model proteins, ribonuclease A, lysozyme, bovine serum albumin and concanavalin A. The hypothesis that silicone oil can induce conformational changes that lead to aggregation was examined by CD and UV spectroscopy. No strong evidence for this was found, and it was suggested that the silicone oil had direct effects on protein intermolecular interactions through interaction with the protein surface.

Silicone adhesion to whole organisms, particularly bacteria, has also been studied for many years.[12] In the most recent publication, Simon Ng and co-workers[13] attempted to produce stable and bacteria-resistant silicone surfaces by varying the degree of hydrophobicity and surface structure. They measured the force necessary to retract an *E. coli*-coated AFM tip from silicone and modified silicone surfaces. The strongest binding was found for unmodified polydimethylsiloxane (PDMS) and the weakest for a fluoroalkylsilyl surface. An octadecylsilyl-modified surface showed intermediate binding properties. This approach is innovative and informative, however, the *E. coli* coat is largely lipopolysacharride (LPS), and additionally *O*-antigen portion adhesion is a major contributor to bacterial adhesion. Comparisons with protein–silicone binding cannot therefore be easily made from this work, although the approach could be extended to protein–silicone interactions.

Studies of the binding of proteins and other biomolecules to silicone surfaces usually suffer from two limitations. Silicone surfaces are difficult to obtain 'clean', and it is sometimes impossible to disentangle silicone binding to proteins from those of surface contaminants. Simon Ng and co-workers[13] overcame this by rigorously cleaning their surfaces, including the use of a plasma cleaner. The second limitation is that bulk silicone materials do not present a large surface for study, and mechanically fragmented materials are frequently used to generate the necessary large surface areas.

Given the importance of silicone–protein interactions and the difficulty of their study, we decided to explore a different approach. The aim of this work was to try to understand, at the molecular level, the nature of the interactions between peptides and silicon compounds that are closely related to silicones. This reduces the complexity of the experiment but introduces approximations and simplifications. However, useful insights can be obtained through model studies.

In this initial study, we used a combination of two model systems to explore the nature of silicone–protein interactions. Phage display technology was used to present a combinatorial library of peptides, in this case PhD.-12, to model silicone surfaces. Solid, molecular cage silsesquioxanes were used as models for silicone surfaces.

The experiment is simple in conception. A library of peptides, attached to the protein coat of phages, is exposed to a solid silsesquioxane. After a brief incubation, the solid is washed with a variety of reagents. After washing, the phages bound to the solid are analysed and the amino acid sequence of the binding peptides is determined. Inferences concerning the nature of the binding may then be made based on the type, distribution and frequency of the amino acid residues in the binding peptides.

Phage display is a random selection technique where a library of peptides, usually 12 or 7 amino acids long, is expressed as a fusion of the protein gIII of M13 bacteriophages.[14] Each individual phage in the library displays a group of identical peptides on its surface. The entire peptide library displays some 10^9–10^{11} different peptide combinations. Screening this library of combinatorial peptides can be used to identify peptides that bind selectively to surfaces.[15,16] There have been very few phage display studies on binding to plastics and polymers.[17]

Phage display has been used to investigate peptide binding to silica.[18,19] In these examples, peptides that bind strongly to silica tend to be heavily enriched in histidine, which reflects the presence of silanol groups on the surface of silica.

Phage display technology generally consists of exposure of the phages to the target, washing out of the non-bound phages, followed by elution of the bound phages, usually with an acid buffer. The eluted phages are then amplified and re-exposed to the target. After three or four rounds of enrichment of the binding clones, the DNA of the phage is sequenced and the binding peptides characterised. In a modification of this method, Stone and co-workers[20] showed that, by using PCR-driven phage display, it is possible to identify some phages that resist the acid washing and remain bound to the target (tight binders), reducing the need to engage in further enrichment rounds, where there is the danger of selective enhancement of phages carrying particular amino acids or combinations of amino acids through evolutionary and other pressures. It also seems possible that washing the substrate with acid could selectively liberate the library phages with the more basic fusion peptides, but this does not appear to have been studied.

A polymerase chain reaction (PCR) is used to detect the presence of peptide-displaying phage DNA. The DNA is then visualised in agarose gels, and it is possible to identify as few as 10–15 phages. An advantage of this modified method is that it is also possible to measure the non-specific binding of the phages to the target. To achieve this, the libraries can be doped with wild-type M13 bacteriophage. Wild-type phages do not contain any additional peptides expressed on the protein coat and, as they lack the fragment of DNA that codifies for the peptides, the PCR product DNA obtained is shorter (250 bp) than that of the peptide-displaying phages (300 bp). Binding to wild-type phages is through the protein coat of the phage, and this mode of binding reveals no accessible useful information.

Cage silsesquioxanes (T^n) have closed cages with $RSiO_{\frac{3}{2}}$ units at each vertex. They are relatively easy to synthesise, can be made with a wide variety of pendant R-groups on the cage and are generally high melting point solids.[21,22] Cage silsesquioxane compounds have received much attention for their applications in materials chemistry.[23–26]

The advantage of working with silsesquioxanes as approximations of silicones is two-fold. First, they are solids and therefore easy to work with. Silicone polymers are frequently gums and liquids before cross-linking, which creates major

difficulties for phage display technology. The second advantage is that they are molecular, easy to purify and there is no ambiguity about the functional groups on the material surface available for binding. Commercial silicone materials are multicomponent and usually contain a filler. We chose the model approach after initial experiments with silicone tubing, which was frozen and crushed to give a finely divided powder with a large surface area. Close analysis of this powder by SEM and other techniques showed it to have a very large amount of silica in very small, high surface area particles. Silica is used as a filler in many silicone materials and can also migrate to the surface, even before crushing. This illustrates the problem of working directly with silicone materials or devices where the composition is not known.

In this work, we used phage display technology to pan against four different silsesquioxane compounds, shown in Fig. 1, octamethyloctasilsesquioxane (methyl^8-T^8), octaphenyloctasilsesquioxane (phenyl^8-T^8), octahydridooctasilsesquioxane (H^8-T^8) and dodecatrifluoropropyldodecasilsesquioxane (trifluoropropyl^{12}-T^{12}). The first two silsesquioxanes bear simple aliphatic (Me) and aromatic (Ph) pendant groups, and are simple silicone analogues. The second two silsesquioxanes have functionalised pendant groups, H and $CF_3CH_2CH_2$. The compounds chosen enable us to examine, at the functional group level, likely interactions between proteins and a variety of silicone materials.

2. Experimental

2.1 Phage display screening

The silsesquioxanes (10–20 mg ml^{-1}) were suspended in tris buffer (pH 7.5), which contained 0.5% of the surfactant Tween 20 and 10^{10} phages from a PhD.-12 library (New England Biolabs), to which 10^9 wild-type phages had been added. This slurry was incubated at room temperature with gentle rotation. After 1 h, the solids were spun at 15 000 rpm and the supernatant, containing the non-bound phage, was recovered. In order to elute the weak binders, the solids were washed 10 times with TBST (tris buffered saline tween 20: tris is tris[hydroxymethyl]aminomethane) 0.5% v/v and once with glycine–HCl buffer (pH 2.2), followed by surfactant washes (50–300 ml). The acid eluate was transferred to a fresh tube and neutralized by adding 150 µl of TBS (tris buffered saline) (pH 9.1). The solids were neutralized by washing twice with TBST (pH 7.0). Strong binders remained bound to the solid. HotStart Taq polymerase master mix (Qiagen) (25 µl), containing HotStart Taq polymerase (5 units µl^{-1}), PCR buffer (Tris-Cl, KCl, $(NH_4)_2SO_4$, 3 mM $MgCl_2$; pH 8.7) and dNTPs (deoxynucleotide triphosphate) (400 µM each), was added to separate aliquots of (a) the non-bound phages, (b) the acid eluate and (c) a slurry of the solids. Forward (5′-CCTCGAAAGCAAGCTGATAAACCG-3′) and reverse (5′-CATTCCACAGACACAACCCTCATAG-3′) primers, and 1 µl of a 100 mg ml^{-1} solution of BSA (bovine serum albumin) were also added to the mixture. The PCR tubes were placed in a thermocycler and heated at 95 °C for 15 min to activate the enzyme and lysate the phages, and were consequently subjected to 35 cycles of denaturisation at 95 °C for 15 sec, annealing at 57 °C for 20 sec and extension at 72 °C for 1 min, with a final extension step of 3 min at 72 °C. 10 µl of each PCR product were loaded into a 3% agarose gel containing ethidium bromide and visualised with a UV lamp.

Agarose gels were run in a standard BioRad gel electrophoresis tank. 1–3% agarose gels were made by suspending the corresponding amount of agarose in a tris-borate EDTA (TBE) buffer and melting it in a microwave oven.

The silsesquioxanes were chemically unchanged by the panning process.

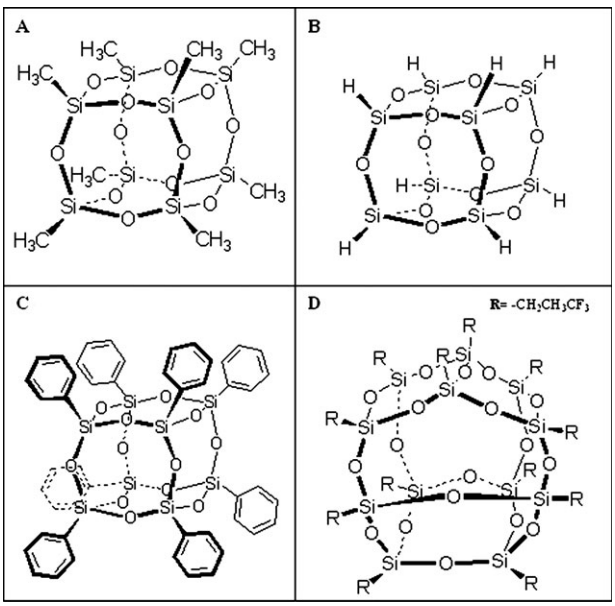

Fig. 1 Cage silsesquioxanes **A**: Methyl^8-T^8, **B**: H^8-T^8, **C**: Phenyl^8-T^8 and **D**: Trifluoropropyl^{12}-T^{12}.

2.2 Cloning of PCR products

The PCR product of interest was first cloned using TOPO vector (Invitrogen). The protocol used was slightly different from the one specified by the supplier. The cloning reaction was prepared by mixing 1 µl of the PCR product, 3 µl of water and 1 µl of a solution of 1.2 M NaCl and 0.06 M $MgCl_2$. The solution was incubated for 5 min at room temperature after the addition of 1 µl of TOPO vector pCR II. Next, 4 µl of the cloning reaction was transferred to a vial of TOP10 competent cells and incubated in ice for another 5 min. After that, 200 µl of SOC medium was added and the culture was shaken for 30 min at 200 rpm and 37 °C. 10, 60 and 130 µl of culture were plated in plates that contained 100 µg ml^{-1} of ampicillin, and incubated overnight at 37 °C.

Single colonies from plates containing less than 100 colonies were analysed using PCR amplification for the presence of the insert.

Colonies that showed the inserted PCR product were used to inoculate LB media containing 50 µg ml^{-1} of ampicillin. Cultures were grown overnight at 37 °C with mild shaking, and plasmid DNA was extracted from pelleted cells using Qiagen miniprep columns. The DNA concentration was estimated by measuring the OD (optical density) at 260 nm.

2.3 DNA sequencing and peptide analysis

Single-stranded DNA isolated from the phage clones was sequenced by using 96 gIII primer (5′-CCCTCA-TAGTTAGCGTAACG-3′) and an automated sequencer (Macrogen, Korea).

The DNA sequences were translated into amino acids by using the "translate" program on the proteomics server of the Swiss Institute of Bioinformatics Expert Protein Analysis System (ExPASy [http://www.expasy.ch/]).

The information content, $-\ln P$ (a measure of the probability, P, of finding each particular peptide), of the selected peptides were calculated by using the bioinformatic server RELIC [http://relic.bio.anl.gov/index.aspx].

2.4 Silsesquioxane and silicone materials

Octamethyloctasilsesquioxane, octaphenyloctasilsesquioxane, octahydridooctasilsesquioxane and dodecatrifluoropropyldodecasilsesquioxane were purchased from Hybrid Plastics. Silicone tubing was donated by Dow Corning, Midland, MI, USA.

3. Results and discussion

Each of the silsesquioxanes was exposed to the phage library and was then washed a number of times, first with acid and then with surfactant. The aim of these repeated and increasingly powerful washes was to remove, as far as possible, the weakest binding clones and leave only tight binders on the solid. The various eluates and the washed solid were subjected to PCR to amplify the phage DNA to measurable levels, and the products were analysed on an agarose gel. In our hands, the agarose gel results gave a qualitative indication of the relative binding abilities of wild-type and library phages to the silsesquioxanes. A referee has pointed out that the PCRs were probably saturated at the concentrations we used. This means that it is not possible to quantitate the agarose gel results, but this does not affect the conclusions we are able to draw.

Our first aim was to probe the binding of PhD.-12 phage peptides to the silsesquioxanes, as compared to wild-type phages. This allowed us to distinguish between random peptide binding from phage coat proteins and specific binding through the expressed peptide. The second aim was to discover the tight binding peptide sequences for each silsesquioxane and to compare these sequences. Similarities and differences between the sequences, as expressed by their amino acid content and distribution, could throw light on the factors affecting protein binding to different silicone materials and devices. By following standard procedures based on Stone and co-workers' PCR method,[20] as described in the Experimental section, tight binding clones were identified and their DNA sequenced. The PCR method was used to amplify the DNA of the phages that remained bound to the silsesquioxanes after acid and surfactant washes. This process enabled the DNA of the phage to be sequenced and the binding peptides to be characterized.

The specific outputs of our chosen method can be illustrated by reference to the results obtained for methyl^8-T^8. The agarose gel (see ESI†) showed that there were many wild-type phages, as well as library phages, bound to the solid after acid elution. The wild-type phages, having no library peptides expressed on the surface, can only bind through the protein coat, and represent non-specific protein binding of the phages to the materials. When the acid-eluted methyl^8-T^8 solid was further washed with 50 ml of tritonX100 0.5% detergent, none of the phages remained bound to the solid.

The results of panning against phenyl^8-T^8 were very similar to those for panning against methyl^8-T^8. Both wild-type and library phages bound to the solid silsesquioxane and resisted washing with acid, but all phages were removed by washing with surfactant. Again, these results are consistent with non-specific binding of phages to the silsesquioxane, most probably through the protein coat of the phages, including the library phages.

Panning against H^8-T^8 and trifluoropropyl^{12}-T^{12} was qualitatively different from panning against the methyl- and phenyl-substituted silsesquioxanes. The relative amount of wild-type phage binding was much reduced, even in the acid eluate and on the acid-washed solid. Furthermore, even after washing the solid with detergent, library phage remained bound to the solid. This strongly indicates that binding of the PhD.-12 phage library to H^8-T^8 and trifluoropropyl^{12}-T^{12} is specific to the expressed peptides on the phage coat.

The first conclusion from the PhD.-12 library panning experiments with silsesquioxanes is that protein binding with silsesquioxanes is readily observable, but, as shown by the addition of wild-type phages, non-specific binding can also be significant. This non-specific binding of proteins can easily be missed in conventional panning experiments where no wild-type phages are present, as the binding of library clones can also take place through the phage's protein coat. In the cases of trifluoropropyl^{12}-T^{12} and H^8-T^8, strong binding clones that remain on the solid after all the wild-type phages have been washed away are most likely to be bound through the expressed peptides and therefore be most revealing about the nature of the specific binding.

Further information was obtained by sequencing the DNA for the tight binding clones of each silsesquioxane. The results are given in full in the ESI.† In some reported examples of panning,[16] a small number of consensus sequences may be discovered. In the examples we studied, there were large numbers of sequences, with a very small number of repeated sequences found.

The number of different clones generated in the construction of a phage-displayed library can range between 10^7 and 10^9, which is a value much smaller than that theoretically possible in a 12mer library (4.1×10^{15}). The different steps involved in the generation of the library also affect its amino acid sequence diversity.[27] There is also sufficient evidence that certain peptides are not compatible with the process of synthesis and the assembly of new phages, and cannot be expressed on the surface of phages.[27–29] During panning, we selected peptides with a particular property, and this property did not necessarily rely on or reflect the amino acid frequencies and distributions imposed in the library's construction. The extent to which panning results in particular peptides being selected, rather than being the result of a random binding, may be inferred from a measurement of the changes in amino acid

frequency and distribution patterns or the sequence diversity in a set of sequences.

RELIC[30] is a relatively new bioinformatics tool that helps with the analysis of peptide sequences observed in a panning process. A total of fifteen software programs that can be used for statistical studies of a population of peptides, searching for weak consensus sequences or performing comparisons of these sequences with proteins found in nature, are accessible through the server. The authors have demonstrated that results obtained using the various RELIC programs are statistically significant for 50 or more peptide sequences. The programs are based on complex statistical methods that combine *ab initio* tools, such as the number of codons for each amino acid, with observed amino acid frequencies in the actual (biased) phage cohort. We used the programs to make broad rather than highly detailed comparisons between the different sets of tight binding phage peptides.

One of the programs (INFO) calculates the information associated with a peptide, depending on its representation in the original library. INFO first uses the AAFREQ program to calculate the frequency distributions at each position of a subset of 50 sequences, randomly extracted from the naïve library. By multiplying the probabilities of each amino acid appearing at each position within the peptide, it is possible to calculate the probability of observing any random peptide. The principle may be stated that the less likely it is to find a peptide sequence in an ensemble of peptides, the more likely it is that peptide was selected for affinity. The selected peptide thus carries information, and for each peptide the program calculates an 'information content' parameter, $-\ln P$, with being P the probability of finding that peptide in the library. The most useful visual information from INFO is obtained by plotting a histogram of a normalised occurrence of peptides in the library against information content. For the library, a more or less symmetrical distribution was obtained between values of $-\ln P$ of 28 and 38, and centred on a value of about 33. The histogram of the binding peptide cohort can be superimposed on that of the original library. There are two likely outcomes. If the two histograms are approximately superimposed, the binding phages closely resemble the library phages, and there is likely to be little specific binding. On the other hand, if the binding cohort histogram has a significant component with information content of greater than about 36, then it may be inferred that there is specific binding to the substrate. The histograms for binding to each silsesquioxane are given in the ESI.†

The INFO histogram for methyl^8-T^8 suggests that there may be some small degree of selection of the phages, but the original distribution of the library is still very similar to that of the unselected library. This is consistent with the observation made from the agarose gel of the PCR products, where both wild-type and PhD.-12 phages bind strongly to methyl^8-T^8, although both can be washed out with surfactant.

The INFO histogram for panning against phenyl^8-T^8 silsesquioxane shows that the abundance and distribution of peptide information closely resembles that for the random peptides, again suggesting that the binding is through a random process. This is consistent with the PCR results, which showed a high non-specific interaction with the phage protein coat, as evidenced by strong binding of the wild-type phage.

The DNA of the bound phages was also sequenced for panning against H^8-T^8 silsesquioxane, and the peptides were again analysed with INFO. In this example, the abundance and distribution of peptide information differed significantly from that for the random peptides. A set of peptides were selected with a higher information content than that in the random library. It is assumed that these peptides were unlikely to be present in a selected population due to chance or to growth characteristics; rather, they were most likely present owing to their affinity for the target. The different distribution of peptides, combined with the observation that the binding peptides have a tendency to be higher in information content, is strong evidence for selective binding to the H^8-T^8 silsesquioxane.

We also applied the INFO program to the sequences of the DNA of phages that were tightly bound to the trifluoropropyl^{12}-T^{12} silsesquioxane, and similar results to those of H^8-T^8 were obtained. This again strongly suggests that the selected phages bound to the silsesquioxane specifically, as was assumed from the results of the PCR.

The PCR results and the histograms of information content against occurrence of the selected peptides demonstrate that the silsesquioxanes with phenyl and methyl substituents bind to peptides, but not specifically. In these examples, the protein coat on the phage is likely to be the major contributor to binding to the silicone. This interpretation is suggested by both the histogram distribution and the presence of wild-type phages amongst the library phages after washing of the solid. The statistical methods and data at our disposal do not allow us to speculate on the nature of these interactions. Some of the peptide fusions may be binding specifically to the methyl^8-T^8 surface, as shown by the slight bias towards high information content peptides. However, it was not possible to disentangle the specific binding from the random in this case. Further analysis of the amino acid sequences for phenyl^8-T^8 and methyl^8-T^8 were not carried out as useful information could not be extracted.

In contrast, most, if not all, of the wild-type phages were able to be washed off, while library phages stayed strongly bound to the silsesquioxanes for panning against both trifluoropropyl^{12}-T^{12} and H^8-T^8. This suggests that the interactions with library peptides are specific to those peptides and depend on their conformations and/or functional groups for efficient binding. The histograms for trifluoropropyl^{12}-T^{12} and H^8-T^8 panning both show binding of silsesquioxanes to high information content peptides.

It was considered useful to analyse the data for trifluoropropyl^{12}-T^{12} and H^8-T^8 panning further using the statistical methods developed by Rodi *et al.*[27] for the diversity profiling of individual amino acids in library peptides. The numbers of amino acids in each of the sequences of library and bound phages may be calculated easily from the data. However, as there is bias in the amino acid frequencies,[27] it is much more revealing to look at the probability of amino acids appearing in particular positions in the PhD.-12 library and comparing these data with that obtained for the binding peptides. The frequencies of amino acid residues observed at each position was calculated using the AAFREQ program (data not shown) for 50 random peptides and 50 peptides selected in the panning

against trifluoropropyl^{12}-T^{12} and H^{8}-T^{8}. To characterize quantitatively the bias as a function of position in these libraries, a measure of how unexpected the observed amino acid frequencies are must be used. To achieve this, we calculated the probability of each amino acid frequency at each position. The results obtained for 50 or more peptides in each cohort are shown in the ESI.† Amino acids with an abundance of at least one standard deviation higher than expected were noted. The profile for the silsesquioxane-binding cohort was compared with that for the library selection, and amino acid residues that appeared at least two standard deviations more frequently than expected were taken as significant. The results for H^{8}-T^{8} show significantly enhanced proline content at positions 7 and 8, enhanced histidine at positions 5 and 9, and enhanced threonine at position 11 (as examples of the actual numbers of amino acid residues involved, the proline residue appears nine times in position 7 and ten times at position 8 in the 52 H^{8}-T^{8} tight binding peptides sequenced). The proline residues presumably induced a favourable conformation in the peptide for binding to the silsesquioxane surface, probably by introducing turns into the peptide chains at critical points. The enhanced histidine content is significant. It has been known for many years that imidazole has a particular affinity for electrophilic silanes, and the interaction can, in favourable cases, lead to pentacoordinate silicon species. Thus, an amino acid bearing an imidazole side chain seems well suited to binding to SiH compounds, in which the silicon is relatively sterically unencumbered and electrophilic.[31–33] It is well known that, under certain conditions and particularly under basic conditions, imidazole catalyses the solvolysis of SiH compounds through pentacoordinate intermediates or transition states. In this case, the SiH bond was unchanged by interaction with the phages. However, the pH of the medium was never higher than the 7.5 of the tris buffer used in the incubation. It is also possible that an SiH centre bound to a phage is highly sterically shielded from attack by any nucleophile, which also militates against SiH cleavage.

The amino acid distribution for trifluoropropyl^{12}-T^{12}-binding peptides is markedly different from that for H^{8}-T^{8} silsesquioxane. The enhanced amino acids are again proline, but in positions 4, 11 and 12, and there are also very high concentrations of serine in positions 1, 9 and 11, and threonine in positions 1, 2 and 12. The highly polar CF_3 group is likely to engage in hydrogen bonding with the OH groups of the serine and threonine side chains, accounting for tight binding to trifluoropropyl^{12}-T^{12} silsesquioxane. It was, to some extent, unexpected that a trifluoro-substituted silsesquioxane would bind to peptides as silicone analogues have very low surface energies, and are resistant to fouling by proteins and other biomolecules (as shown by Simon Ng *et al.*[13] and others). It was not possible, through these experiments, to determine the relative strengths of binding to peptides, and the high information content peptides that bind to the trifluoro-substituted silsesquioxane may not necessarily be analogous to those commonly found in biological environments. It was noted that the non-specific binding, as demonstrated by binding to wild-type phages, is weak for trifluoropropyl^{12}-T^{12} silsesquioxane, consistent with a low surface energy.

4. Conclusions

In conclusion, the combination of phage display technology and the use of silsesquioxanes as models has been shown to be useful in analysing and modelling the interactions of proteins with silicones.

The observations are consistent with our knowledge of silicone chemistry and the nature of weak interactions. Overall, the phage display methodology has proved useful in probing silicone–protein interactions, and the use of silsesquioxanes as surrogate silicones appears, at least in part, to be justified. These relatively unfunctionalised silicones have been confirmed to bind to proteins through generalised, non-specific polar interactions, probably in the manner described by Brook *et al.*[5] Functionalised silicones, by way of contrast, are likely to bind more specifically—and more strongly, as shown by the panning experiments—through their functional groups interacting with protein side chains. The judicious positioning of proline residues is likely to facilitate tight binding by bringing functional groups into appropriate positions for interaction.

Si–H functional materials bind to proteins, notably through interaction with histidine residues. In this example, the accessibility of the silicon atom for interaction with the imidazole ring probably gives complexes with pentacoordinate or equivalent silicon centres. Although silicon has a well-established affinity for imidazole groups, it is surprising that other nucleophilic and potentially coordinating groups, such as NH_2 or OH, are not heavily represented in the amino acid side chains of peptides binding to SiH-containing silicones. The trifluoropropyl-substituted silicones also bind proteins and, in this case, the main binding mode appears to be hydrogen bonding to the OH groups in serine and threonine residues.

Further experiments could be carried out to compare molecular silsesquioxanes with silicone homopolymers bearing equivalent side chain groups. This would be most interesting for the fluoro-substituted compounds. However, these homopolymers are not easy to prepare in a useful solid form, apart from dimethylsilicone, and it is not clear that much more useful information could be gained from what would be a time consuming and even more difficult study than the current work.

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