Self-assembled multivalent RGD-peptide arrays – morphological control and integrin binding†

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We report the synthesis of four different RGD peptide derivatives which spontaneously self-assemble into nanoscale architectures. Depending on the information programmed into the molecular-scale building blocks by organic synthesis, these compounds assemble into different nanoscale morphologies. This process can be fully understood using multiscale modelling which provides predictive insight into subtle differences, such as whether the compounds form spherical micelles, rod-like cylinders or tubular assemblies, and predicts experimentally observed critical aggregation concentrations (CACs). We then probe the multivalent binding of these assemblies to integrin proteins and demonstrate that the spherical micellar assemblies perform well in our solution-phase integrin binding assay as a consequence of self-assembled multivalency, with the CAC switching-on the binding. Conversely, the cylindrical assemblies do not work in this assay. As such, the nanoscale morphology controls the apparent ability to perform as a self-assembled multivalent ligand array.

Introduction

Multivalent binding is a powerful strategy to achieve high-affinity molecular recognition between nanoscale surfaces. In recent times, there has been increasing attention on the use of self-assembly methods to generate nanoscale multivalent arrays. Self-assembly is a synthetically straightforward strategy, in which monovalent building blocks spontaneously form higher order architectures. Using self-assembly to generate multivalent ligand arrays offers a number of advantages over the covalent construction of such systems: (i) spontaneous simple assembly, (ii) well-defined low-molecular-weight building blocks suitable for clinical approval, (iii) easily tunable ligands, (iv) tunable nanostructure morphologies, (v) ability to assemble different active components into a single nanostructure, (vi) simple or triggered disassembly/degradation. By tuning the choice of ligands it is possible to modify these self-assembled multivalent systems to bind a range of different biological targets, for example multivalent sugar arrays can bind lectins and hence interfere with viral adhesion. Multivalent cationic arrays can bind polyanions such as DNA and heparin, with applications in gene delivery and post-surgical therapy. In this way, self-assembled multivalency (SAMul) is a viable approach for the generation of new synthetic solutions to nanomedicine.

Given the importance of integrin proteins on cell surfaces, there has been intense interest in studying the interaction between RGD peptides and peptidomimetics with this class of protein. Such interactions are vital in mediating the ways in which cells interact with the extracellular matrix, and have therefore been employed in materials with potential applications in tissue engineering. Furthermore, the optimisation of such interactions has been proposed as a way of targeting tumour cells which have up-regulated levels of integrin proteins. On initial inspection, integrins may appear to be unpromising targets for multivalent binding, as they only possess a single ligand binding site. However, in biological systems, integrins are often clustered in cell membranes. To enhance the binding of RGD peptides to integrins, there has therefore been interest in using multivalent peptide arrays. Self-assembled arrays of RGD peptides have been reported in the literature. RGD-containing lipopeptides have been demonstrated can be insert into membranes and liposomes which gain enhanced affinity for integrins and/or show modified biological properties. Self-assembling RGD-functionalised lipids have also been shown to assemble into morphologies such as nanoribbons or fibres. Given the importance of RGD-integrin interactions, we have recently been interested in gaining a fundamental insight into the nature of multivalency in RGD...
peptide–integrin interactions and demonstrated that self-assembled arrays of RGD peptides act in a multivalent manner (Scheme 1) analogous to the way in which dendritic molecules can arrange multiple ligands, enhancing the interaction with integrin proteins within vesicle membranes.\textsuperscript{15}

In this new paper we demonstrate (i) how the self-assembled approach to multivalency can easily yield a range of different nanoscale multivalent structures, (ii) how this precise morphological control can modify interactions with the biological target. Furthermore, using multiscale modelling we gain an understanding of subtle effects in the self-assembly process. This exemplifies how a combined experimental and theoretical approach to self-assembled multivalency will be a powerful way of designing and developing novel morphologically tunable nanoscale systems capable of intervening in biomedical processes.

**Results and discussion**

**Synthesis and characterisation**

For the purposes of this study, we targeted a series of compounds in which an RGD peptide unit was attached to different hydrophobic groups (Fig. 1) – we intended to demonstrate the impact of the hydrophobic (self-assembling) part of the molecule on self-assembly.

Compound C12-RGD, synthesised by TBTU-mediated coupling of lauric acid to a protected RGD peptide, followed by global deprotection, was reported by us previously.\textsuperscript{15} Using the same general synthetic methodology, we coupled behenic acid or 1-pyrenebutyric acid to the same peptide, and generated C22-RGD or Py-RGD in very good yields of 63% and 77% respectively (see ESI † for details). We also designed a larger twin-tailed hydrophobic unit for conjugation to the RGD peptide to give (C12)\textsubscript{2}Lys-RGD. This compound was synthesised according to the method outlined in Scheme 2. The hydrophobic unit was synthesised by TBTU-mediated conjugation of lauric acid to lysine methyl ester to provide compound \textbf{1-ester}. Basic saponification unmasked the carboxylic acid to give \textbf{1-acid} which was then coupled with methyl 6-aminohexanoate, again by TBTU-mediated peptide coupling methods to give compound \textbf{2-ester}. Saponification of the methyl ester provided \textbf{2-acid}. The overall yield for this four-step synthesis was a pleasing 46%. We then needed to attach a hydrophilic tri-ethylene glycol unit to the RGD peptide in order to provide the target compound with sufficient solubility in water (this was discovered experimentally). This was achieved by attaching compound \textbf{3-acid} to the N-terminus of the globally protected RGD peptide to give compound \textbf{4} in 52% yield. Reduction of the azide to the primary amine using \textit{H}\textsubscript{2} with \textit{Pd/C} proceeded to provide compound \textbf{5} in quantitative yield. Finally, coupling compound \textbf{5} to compound \textbf{2-acid} via TBTU-mediated peptide coupling, followed by global

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig1.png}
\caption{Compounds investigated for self-assembly and integrin binding in this paper – C12-RGD, Py-RGD, C22-RGD and (C12)\textsubscript{2}Lys-RGD.}
\end{figure}
deprotection gave target compound \((\text{C}12\text{)}_2\text{Lys-RGD})\) in an acceptable yield of 11%.

**Self-assembly of RGD derivatives**

The self-assembly of the four target compounds was initially investigated via a Nile Red assay – in this experiment, Nile Red only becomes solubilised into water (pH 7.4) at concentrations above the critical aggregation concentration (CAC). Monitoring the fluorescence emission of Nile Red \((\lambda_{\text{ex}} = 550 \text{ nm}, \lambda_{\text{em}} = 650 \text{ nm})\), and plotting the intensity against \(\log[\text{RGD-derivative}]\) (see ESI†) allows the CACs to be determined (Table 1). Under these pH conditions, the compounds investigated should have one net negative charge on the RGD unit (the arginine side chain is cationic, while the aspartic acid is anionic on both its side chain and its terminus).

Interestingly, \(\text{Py-RGD}\) had a significantly lower CAC \((110 \mu\text{M})\) than \(\text{C}12\text{-RGD} (295 \mu\text{M})\). We suggest that \(\pi-\pi\) interactions and packing effects between pyrene groups may reinforce the self-assembly of these hydrophobic units. In order to confirm this suggestion, we carried out variable concentration experiments on \(\text{Py-RGD}\) in order to probe the inherent fluorescent emission from the pyrene units \((\lambda_{\text{ex}} = 343 \text{ nm}, \lambda_{\text{em}} = 380–440 \text{ nm})\). On increasing the concentration of \(\text{Py-RGD}\) the fluorescence emission spectrum decreased in intensity, indicating that self-assembly of pyrene in the core of the self-assembled nanostructures results in fluorescence quenching. Plotting the monomer emission \((382 \text{ nm})\) against \([\text{Py-RGD}]\) indicated that at ca. 120 \(\mu\text{M}\), there was a discontinuity (see ESI† for data) – this reflects the CAC value, at which assembly into nanostructures becomes favoured. Therefore, the data collected using the inherent fluorescence of \(\text{Py-RGD}\) was in general agreement with the Nile Red ‘external dye’ approach.

On further increasing the extent of hydrophobic functionalisation, the CAC changed by an order of magnitude, with self-assembly becoming significantly more favoured. For compound \(\text{C}22\text{-RGD}\) the CAC was determined as 30 \(\mu\text{M}\) using the Nile Red assay – a ten-fold reduction of CAC compared with \(\text{C}12\text{-RGD}\), indicating the effect of extending the hydrophobic chain length. In our previous work on polycationic self-assemblies for DNA binding, we have also noted that increasing the hydrophobic chain length from \(\text{C}12\) to \(\text{C}22\) induced a change in CAC in a similar way, although even more dramatically from 210 \(\mu\text{M}\) to 2 \(\mu\text{M}\).\(^5\) We suggest that the magnitude of the effect of hydrophobic functionalisation on the CAC of self-assembled systems depends on the amount of charge on the head group which has to be brought into close proximity on the surface of the self-assembled nanostructure.

Finally, twin-tailed \((\text{C}12\text{)}_2\text{Lys-RGD})\) exhibited highly effective self-assembly, with a very low CAC of 6 \(\mu\text{M}\), once again indicating that increasing the degree of hydrophobic functionalisation drives increased propensity for aggregation in aqueous solution. The thermodynamics of self-assembly were explored further using multiscale modelling methods (see below).

**Table 1** Critical Aggregation Concentration (CAC) values determined by Nile Red encapsulation assay

<table>
<thead>
<tr>
<th>Compound</th>
<th>CAC value/(\mu\text{M})</th>
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<tbody>
<tr>
<td>(\text{C}12\text{-RGD})</td>
<td>295</td>
</tr>
<tr>
<td>(\text{Py-RGD})</td>
<td>110</td>
</tr>
<tr>
<td>(\text{C}22\text{-RGD})</td>
<td>30</td>
</tr>
<tr>
<td>((\text{C}12\text{)}_2\text{Lys-RGD}))</td>
<td>6</td>
</tr>
</tbody>
</table>

**Scheme 2** Synthesis of \((\text{C}12\text{)}_2\text{Lys-RGD})\).
Morphology of self-assembly

The pioneering work of Israelachvili states that the hydrophilic/lipophilic balance of surfactant-like compounds controls the morphology of their self-assembly,17 and we therefore probed the morphology of our self-assembled nanostructures using both experimental and theoretical methods.

Transmission electron microscopy was used to visualise the nanoscale assemblies (Fig. 2). In each case, solutions of the self-assemblies were dried on the TEM grid, negatively stained with uranyl acetate and then visualised. TEM is a useful comparative technique for families of related molecular structures (as in this case). Compound C12-RGD exhibited clusters of micellar objects when observed by TEM. These micelles were of ca. 5 nm diameter, which is in accordance with the head-to-tail molecular length of this compound, which is ca. 2.5 nm. Interestingly, if C12-RGD was dried from a more dilute solution (just above the CAC), these micelles were not observed, and this may indicate that the self-assembled nanostructures have relatively low stability in this case. Similarly, compound Py-G2 also exhibited clustered micellar assemblies. However, unlike for C12-RGD, if Py-RGD was dried from just above its CAC value, micelles were still observed, which is suggestive of these self-assembled structures having greater stability.

Compound C22-RGD exhibited a completely different morphology with cylindrical assemblies being proposed as the dominant assembly mode (see computational modelling below for further information). However, the dimensions of the observed objects were ca. 20 nm – in contrast to the molecular-scale diameter of ca. 4.1 nm. There was some evidence that these objects were hollow, but also in some places cylinder bundling was possible. In order to understand this morphology more fully, multiscale modelling was required (see below for comments). Clearly, however, extension of the hydrophobic chain has changed the mode of self-assembly in agreement with the work of Israelachvili,17 who noted that if the hydrophobic balance of the molecule increases there is no longer sufficient ‘space’ within a spherical micelle to accommodate the hydrophobic unit.

Interestingly, C22-RGD formed gels in d6-DMSO – it is well-known that systems which can self-assemble into fibrillar nanostructures can form self-supporting networks which underpin supramolecular gels if the solvent is well-chosen.18 In this case, although a gel formed in d6-DMSO, the system did not gel in aqueous solution, however, there was increased viscosity.

Twin tailed compound, (C12)2Lys-RGD, was also observed to form a rod-like self-assembly (Fig. 2D). In this case, however, isolated rods could be observed (Fig. 3). It should be noted that the SEM image (Fig. 2D) is dried from a significantly more concentrated solution than the TEM image in Fig. 3A, and as such, the apparent network densities are somewhat different in the two images. The rods were monodisperse with diameters of ca. 11.5 nm. Given that the extended head-to-tail molecular length is ca. 6 nm, we proposed that the structures observed by TEM are probably simple cylindrical micelles in this case.

In order to gain further insight into self-assembly we went on to perform computational modelling. Modelling has previously been applied by other groups to understand the self-
assembly of RGD peptide-amphiphiles. Full details of computational methods can be found in the ESI,† but the methods can be summarised as follows. Initially we optimized the structures of the individual molecules using AMBER 1120 and the GAFF force field21 in a TIP3P water box22 at proper pH and ionic strength using molecular dynamics methods. The SHAKE algorithm was employed,23 with the Berendsen coupling algorithm providing temperature control24 and the particle mesh Ewald method to treat long range electrostatics.25 Well established procedures were then used to calculate the interaction energies within the solvated system.26 We subsequently parameterised the molecules in order to apply mesoscale dissipative particle dynamics (DPD) methods27 and hence probe their self-assembly behaviour on the nanometre scale. The DPD model captures the essentials of mass and momentum conservation which are responsible for the hydrodynamic behaviour of a fluid at large scales and treats the molecules as a series of beads connected together by Hookean springs. Further details of the number and choice of beads and methods employed to determine these5d,28 can be found in the ESI.† Electrostatic interactions between beads were analysed following the approach outlined by Groot,29 and solvent molecules were also simulated. The characteristic time scale for the systems was then defined matching the experimental and simulated diffusivity for water molecules.30 As such the modelling approach applied is multiscale, ideal for simulating self-assembled nanostructures in solution, and includes aspects of both molecular dynamics and mesoscale modelling.

We began our DPD-based simulation by monitoring the molecular self-assembly processes to gain an insight into the aggregates formed. Fig. 4 summarises the results of the modelling and shows that all hydrophobically-modified RGDs aggregate into supramolecular structures, with different morphologies dependent on the precise molecular architecture. Specifically: (i) C12-RGD and Py-RGD assemble into spherical micelles, with average diameter ($D_m$) and aggregation number ($N$) of 4.6 nm and 52 for C12-RGD and 4.1 nm and 27 for Py-RGD, respectively, (ii) the double-tailed compound (C12)2Lys-RGD self-assembles into an extended cylindrical micelle with $D_m = 9.8$ nm and $N = 3200$, and (iii) the long hydrophobic chain-bearing amphiphile C22-RGD generates rod-like vesicles characterized by a micellar diameter value ($D_m$) of 23.1 nm and an aggregation number (N) of 3050. As such, there appears to be an excellent degree of agreement between the theoretical (Fig. 4) and experimental (Fig. 2 and 3) observations. In particular, the ability of modelling to shed light on the rod-like vesicle morphology formed by C22-RGD is particularly noteworthy. The first two columns of Table 2 summarize these geometrical findings in numerical form.

Considering the origins of this effect in more detail, two opposing forces control self-assembly: hydrocarbon–water interactions that favour aggregation, and head group
interactions that work in the opposite sense. These two contributions can be considered as an attractive interfacial tension term due to hydrocarbon tails and a repulsion term depending on the nature of the hydrophilic group. This basic idea was originally quantified by Israelachvili, Mitchell and Ninham,\textsuperscript{17} resulting in the concept that aggregation of surfactants is controlled by molecular geometry.

The packing parameter, \( P \),\textsuperscript{17} is a very useful parameter which allows the prediction of aggregate shape and size; as is well-known, \( P \) values < 0.33 lead to spherical or ellipsoidal micelles, when 0.33 < \( P \) < 0.5 large cylindrical or rod-shape micelles are predicted, while flexible bilayer structures and vesicles are predicted when 0.5 < \( P \) < 1 and, lastly, reversed or inverted micelles appear for \( P > 1 \). Simple modelling considerations allowed us to calculate the packing parameter \( P \) for all of the modified RGD compounds in hydrated conditions, as shown in the last column of Table 2. Pleasingly, the calculated \( P \) values are in agreement with the more detailed multiscale modeling studies (and experimental observations) about the precise way in which the hydrophobic tails control the ultimate self-assembled morphology of these RGD derivatives.

From an energetic standpoint, the change in Gibbs free energy of transfer of a single amphiphilic molecule from the monomeric state to a micelle of aggregation number \( N \), commonly called the free energy of micellisation \( \Delta G_m \), can be modelled as consisting of a hydrophobic part, \( \Delta G_{m,h} \), and an electrostatic part, \( \Delta G_{m,e} \), so that \( \Delta G_m = \Delta G_{m,h} + \Delta G_{m,e} \). The value \( \Delta G_{m,h} \) is essentially the free energy associated with transferring hydrocarbon chains out of water and into the oil-like interior of the micelle (a favourable contribution), plus the surface free energy attributed to solvent-hydrocarbon contacts in the micelle (an unfavourable contribution). The second term, \( \Delta G_{m,e} \), originates from unfavourable contributions associated with head group interactions, including electrostatic as well as head group conformation effects. We calculated the values of \( \Delta G_m \) for the four RGD-derivatives as shown in the first column of Table 3. As expected, \( \Delta G_m \) at room temperature and physiological ionic strength, has large, negative values, indicating that micellisation is a spontaneous and highly favourable process for all compounds. Interestingly, however, the predicted values of \( \Delta G_m \) decrease on going from C12-RGD to C22-RGD and, since the headgroup architecture is the same in all amphiphiles, the main differential contribution to \( \Delta G_m \) stems from the \( \Delta G_{m,h} \) term which, in turn, must reflect differences in the size and structure of the hydrophobic component.

The critical micelle concentration (CMC) is one of the most commonly studied properties of a self-assembled system because it is a direct measure of the thermodynamic stability of the micelles in solution. Basic thermodynamic relationships allow CMC to be directly obtained from \( \Delta G_m \); in particular, for ionic micelles in a solution of high electrolyte content as in the present study, we have:

\[
\Delta G_m \approx RT \ln(\text{CMC})
\]  

The second column in Table 3 shows the calculated CMC data for each system simulated in this work. Typically, micellar aggregates have CMCs of the order of \( 10^{-3} \text{–} 10^{-2} \) M, while lower CMCs are found for amphiphiles that form membranes or cylindrical aggregates. This is, at least in part, a consequence of the fact that amphiphiles with low CMCs tend to have relatively large hydrophobic segments, and this normally results in an assembly shape with a lower curvature. Although a word of caution is due about the fact that the calculated values of \( \Delta G_m \) and CMC are obtained using simplified (but validated) theoretical approaches, importantly the trends exhibited by these parameters are pleasingly in-line with the CAC experimental data (compare values in Tables 1 and 3).

The most important aspect of our simulation approach is the fact that the geometrical/energetical values of the different micellar entities predicted by our computational recipe are in excellent agreement with the corresponding values stemming from experimental evidence (TEM, CAC); confirming the relevance of multiscale molecular modeling and simulation in the study of self-assembly of complex molecular entities. Last but certainly not least, modelling strongly suggests that, for C22-RGD, the fibres observed by TEM actually consist of hollow lamellar-type assemblies rather than bundles of individual fibrils. In this sense, the modelling provides additional insight into the mode of self-assembly which was difficult to obtain using the experimental methods employed, once again demonstrating the synergy between these approaches.

**Integrin binding studies**

We then went on to monitor the binding of these self-assemblies to integrin at pH 7.4, using a fluorescence polarisation (FP) assay, as previously reported by others and ourselves.\textsuperscript{15,31} In this assay, integrin \( \alpha_\beta_\text{III} \), dispersed in Triton X-100, is bound to a fluorescent probe molecule (a fluorescein-modified cyclic RGD peptide synthesised in-house).\textsuperscript{15} The compound of interest is then introduced, and if it binds to the integrin, the fluorescent probe is displaced, which can be detected by a decrease

**Table 2** Values of micellar diameter \( D_m \) (nm), core radius \( R_c \) (nm), aggregation number \( N \), and packing parameter \( P \) for the different modified RGD compounds as obtained from DPD simulations

<table>
<thead>
<tr>
<th>Compound</th>
<th>( D_m ) (nm)</th>
<th>( R_c ) (nm)</th>
<th>( N )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>C12-RGD</td>
<td>4.6 ± 0.2</td>
<td>1.4</td>
<td>52</td>
<td>0.32</td>
</tr>
<tr>
<td>Py-RGD</td>
<td>4.1 ± 0.1</td>
<td>1.2</td>
<td>27</td>
<td>0.30</td>
</tr>
<tr>
<td>C22-RGD</td>
<td>23.1 ± 0.5</td>
<td>—</td>
<td>3050</td>
<td>0.80</td>
</tr>
<tr>
<td>(C12)\textsubscript{2}Lys-RGD</td>
<td>9.8 ± 0.4</td>
<td>2.2</td>
<td>3200</td>
<td>0.43</td>
</tr>
</tbody>
</table>

**Table 3** Predicted free energy of micellisation \( \Delta G_m \) (kJ mol\(^{-1}\)) and critical micelle concentration CMC (\( \mu \)M) for the different modified RGD compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>( \Delta G_m )</th>
<th>CMC (( \mu )M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C12-RGD</td>
<td>−10.37</td>
<td>157</td>
</tr>
<tr>
<td>Py-RGD</td>
<td>−11.00</td>
<td>92.2</td>
</tr>
<tr>
<td>C22-RGD</td>
<td>−10.73</td>
<td>21.3</td>
</tr>
<tr>
<td>(C12)\textsubscript{2}Lys-RGD</td>
<td>−13.70</td>
<td>9.4</td>
</tr>
</tbody>
</table>
in the FP signal associated with the enhanced mobility of the probe. This assay is a useful way of comparing related ligands, and gaining a degree of quantitative insight into their interactions with integrin.

As previously reported,\textsuperscript{15} C12-RGD showed a good affinity for integrin α\textsubscript{β}\textsubscript{3} in this assay, and reduced the FP signal below 50% of its initial level at a concentration of 200 μM (Fig. 5). We therefore define the EC\textsubscript{50} as 200 μM for this ligand. This is significantly more effective than a non-self-assembling RGD peptide, as we reported previously which was unable to displace 50% of the probe from the integrin binding site. In order to ensure that any effect on the process was not simply due to the surfactant interfering with the Triton X-100 present in the assay through a detergency effect, we used sodium dodecyl sulphate (SDS) as a negative control. This compound led to no change in the FP signal demonstrating that the presence of the RGD peptide was essential, indicative of specific interactions between the self-assembling peptide and the integrin protein binding site.

We then tested Py-RGD in the same assay. This compound exhibited an EC\textsubscript{50} for integrin binding of 110 μM (Fig. 5). Once again, this is evidence for a self-assembled multivalency effect on binding with the micellar assembly being able to bind to integrin proteins in a more effective way. Intriguingly, the EC\textsubscript{50} value for Py-RGD is very similar to its CAC, and we suggest that only once the peptide assembles can really effective binding be observed. We note, in agreement with this hypothesis, that Py-RGD is a more effective integrin binder than C12-RGD and that the formed compound also has a lower CAC value, and hence will more easily be able to express self-assembled multivalent binding.

We then went on to test the ability of C22-RGD and (C12)\textsubscript{2}Lys-RGD to interact with integrin α\textsubscript{β}\textsubscript{3} in the same assay. These compounds were unable to effectively bind to the protein and exhibited either no binding (C22-RGD) or behaved like a very weakly binding monovalent RGD peptide (((C12)\textsubscript{2}Lys-RGD). It is possible that in these cylindrical-type aggregates, the RGD peptide ligands are less-accessible on the surfaces of the cylinders. Interestingly, Lee and co-workers previously reported that nanostructure morphology could affect the binding of multivalent mannose units to concanavalin A, with small spherical micellar aggregates being shown to have more effective binding to the target than cylindrical aggregates.\textsuperscript{4d} Stupp and co-workers have also previously reported that the mobility and accessibility of peptide ligands on self-assembled multivalent surfaces is important in controlling their ability to bind to biological partners.\textsuperscript{14e} These previous observations would therefore appear be in agreement with our observations as spherical micelles might, as a consequence of their geometry, be expected to provide more space on their surfaces for each individual ligand to bind to its biological partner (according to Israelachivili’s geometric considerations). Alternatively, it is possible that the higher aggregation numbers of the cylindrical aggregates (see Table 2) lowers the on-off rate of binding at their surfaces.

However, it should also be noted that in both cases, the viscosity of the solution increased on adding these cylindrical assemblies, which have a tendency towards the formation of entangled gel-phase network. Fluorescence polarisation assays are highly sensitive to viscosity increases, and so this may also explain why the cylindrical nanoscale morphologies are not as effective as the spherical micelles in this integrin binding assay. Fig. 6A is a TEM image of the Triton X-100-integrin

![Fig. 5](Image) Data from integrin binding assay showing effect of RGD-derivatives on the fluorescence polarisation signal of a fluorescent probe bound to integrin α\textsubscript{β}\textsubscript{3} in Triton X-100. Orange = C12-RGD, Blue = Py-RGD, Red = C22-RGD, Purple = (C12)\textsubscript{2}Lys-RGD. All samples were incubated at 29 °C for 5 min.

![Fig. 6](Image) TEM images of A: Triton X-100/Integrin, scale bar = 500 nm; B: Self-assembled fibres of C22-RGD in the presence of Triton X-100/Integrin, scale bar = 5 μm. Image 6A reproduced from ref. 15.
system used in the assay, and Fig. 6B shows the cylindrical micelles formed by C22-RGD at the end of the integrin binding assay. The Triton X-100/integrin assemblies some of which can be seen tangled up within the fibrous network formed by C22-RGD.

Nonetheless, we conclude that for effective self-assembled multivalent solution-phase binding of integrin proteins in this chemical assay, it is preferable to have spherical micellar nanoscale assemblies, rather than their cylindrical equivalents. In this way, the nanoscale morphology programmed in by self-assembly controls the outcome of the binding assay. Of course, it should be noted that in biological systems, these binding effects may differ, as the integrin proteins will be displayed on cell surfaces in a very different manner to the surfactant-immobilised proteins within this assay. Clearly this is worthy of further study. However, this paper clearly illustrates the impact the effect which morphology can have on nanoscale binding events.

Conclusions

We have reported the synthesis of four different self-assembling RGD-peptide derivatives. Dependent on the choice of the hydrophobic unit, the critical aggregation concentration can be varied over several orders of magnitude, and the nanoscale morphology into which the peptides assembly can be controlled to be spherical, cylindrical or tubular. Multiscale modelling provided direct insight into the self-assembly of these ligands and predicted the precise degree of morphological control, as well as predicting their critical aggregation concentrations. The multiscale modelling allowed us to dissect the thermodynamic terms responsible for self-assembly, and locate the origin of the morphological control firmly in the nature of the interactions between the hydrophobic units within the self-assembled nanostructures.

On studying the ability of these self-assembled nanostructures to bind in a multivalent manner to integrin proteins, it was found that the spherical micellar systems were far more effective in our chemical solution-phase integrin binding assays, than their cylindrical analogues. Furthermore, they were much more effective than a non-self-assembling analogue. Interestingly, the integrin binding behaviour of the spherical micelles only appeared to become effective once the compound was above its critical aggregation concentration – providing further evidence for the conclusion that self-assembly plays a vital role in enabling high-affinity integrin binding – self-assembled multivalency (SAMul). The cylindrical, rod-like assemblies led to viscosification, and appeared unable to displace the fluorescent probe from the integrin binding site. As such, this paper indicates the importance of optimising nanoscale morphology and dynamics using a combined experimental and theoretical methodology when generating SAMul ligand arrays, and clearly demonstrates the versatility and controllability of this approach. Future work will focus on investigating the performance of different morphologies in a biological setting and developing more detailed structure–activity relationships for SAMul binding arrays.

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Notes and references


