Experimental report

24/06/2017

Proposal: 9-10-1412 **Council:** 10/2014

Title: Elucidation and control over the self-assembly of (cyclic peptide)-polymer conjugates

Research area: Chemistry

This proposal is a new proposal

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Samples: CP-(p(n-butyl acrylate)30)2 in DMSO-d6
CP-(p(n-butyl acrylate)30)2 in CDCl3
CP-(p(n-butyl acrylate)30)2 in THF-d8
CP-(p(n-butyl acrylate)30)2 in TFA-d
NMeCP in THF-d8
NMeCP-p(n-butyl acrylate)16 in THF-d8

Instrument	Requested days	Allocated days	From	To
D11	2	3	04/08/2015	06/08/2015
			10/07/2016	11/07/2016

Abstract:

The self-assembly of (cyclic peptide)-polymer conjugates has recently been exploited to form functional, rigid nanotubes. Overshadowed by the numerous applications, the fundamental understanding of the self-assembly mechanism remains undetailed, yet such studies and findings will allow predictions to the length of the nanotubes and furthermore implicate the development of methods that could allow control over this important dimension. Here we propose several experiments to elucidate and influence the self-assembly, using small angle neutron scattering to follow the changes to the nanostructure.

Elucidation and control over the self-assembly of (cyclic peptide)-polymer conjugates – Experiment 9-13-668

The project explored the self-assembly of (cyclic peptide)-polymer conjugates as a versatile supramolecular motif to engineer nanotubes with defined structure and dimensions. This work expands on the theoretical model of single step nucleation-elongation growth of self-associating species, by showing that the degree of polymerization decreases when monotopic species interact sufficiently strongly with the ditopic assembly. We also show that the introduction of monotopic species provides the opportunity to introduce a high percentage of end-functionalised assemblies through a cooperative self-assembly mechanism.

We studied experimentally the addition of partially *N*-methylated cyclic peptides (^{NMe}CPs) (**Figure 1**) as monotopic species to a solution of (cyclic peptide)-polymer conjugates that self-assembles to form rigid nanotubes. By alternating *N*-methylated amino acids in the peptide sequence, cyclic peptides can be engineered to have directed H-bonding restricted to one face.[1] It has been found that ^{NMe}CPs and CPs can associate together as evidenced by previous work by Ghadiri *et al*. (as mentioned earlier) where ^{NMe}CPs were used to modulate the flux of ions through cyclic peptide based channels.[2] We envisaged a binary mixture of ^{NMe}CP species and CP species in solution, where the ^{NMe}CP species would present itself at the ends of the nanotube assembly and behave as 'chain stoppers'. In this case, assuming $K_2 = 2000\ M^{-1}$ and $K = 200000\ M^{-1}$ at 1 mM, the number average length of the nanotube assembly is expected to decrease from 45 nm to 5 nm with 10 mol% chain stoppers, and as short as 1.5 nm with 50 mol% chain stoppers, provided that adequate association exists between the monotopic and ditopic species.

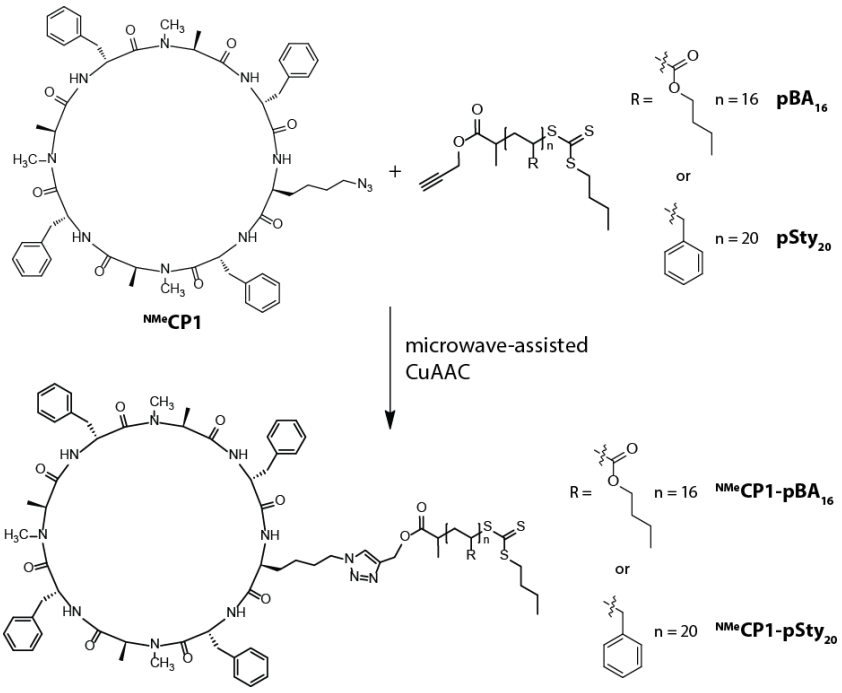

Fig. 1. Structure overview of *N*-methylated cyclic peptide **^{NMe}CP1** and (*N*-methylated cyclic peptide)-polymer conjugates **^{NMe}CP1-pBA$_{16}$** and **^{NMe}CP1-pSty$_{20}$**.

We synthesised cyclo[-L-Lys(N_3)-D-Phe-(*N*-Me-L-Ala-D-Phe-)$_3$] **^{NMe}CP1**, and conjugates **^{NMe}CP1-pBA$_{16}$** and **^{NMe}CP1-pSty$_{20}$** (**Figure 1**), which show H-bonding and dimerization, despite their

propensity to aggregate in solution beyond the directional antiparallel *β*-sheet motif (forming large (400 nm) structures in the case of the conjugates).[3] By small angle neutron scattering we observed the scattering profiles of mixtures of **^{NMe}CP1**, **^{NMe}CP1-pBA$_{16}$** and **^{NMe}CP1-pSty$_{20}$** with **CP1-(p(BA-d_9)$_{29}$)$_2$** conjugates in THF-d_8 in molar ratios of 1:1, 1:2, 1:4 ([CP]:[^{NMe}CP]) keeping the concentration of the ditopic species constant at 1 mM. Large structures were observed to form by the ^{NMe}CP conjugates, and it was necessary to manipulate the data to filter the contribution of scattering in order to probe the nanotube assembly. Subtracting the scattering intensities of the control experiments (^{NMe}CP species and with no CP conjugates in THF-d_8) was a straightforward manipulation that yielded a reasonable set of data to interpret. The resulting scattering profiles (**Figure 2**) showed no change to the geometry of the nanotube assembly, even at 80 mol% monotopic species. We have previously observed lateral aggregation of **^{NMe}CP1** in solution,[3] such aggregation reduces the availability of monotopic species that are able to bind to ditopic species, and can be crudely represented by monotopic species with a very high affinity to dimerise compared to the hetero-association between monotopic and ditopic species. In this description, our theoretical model identifies a decreased perturbation of the assembly length, showing that **^{NMe}CP1** as an inefficient chain stopper for **CP1**.

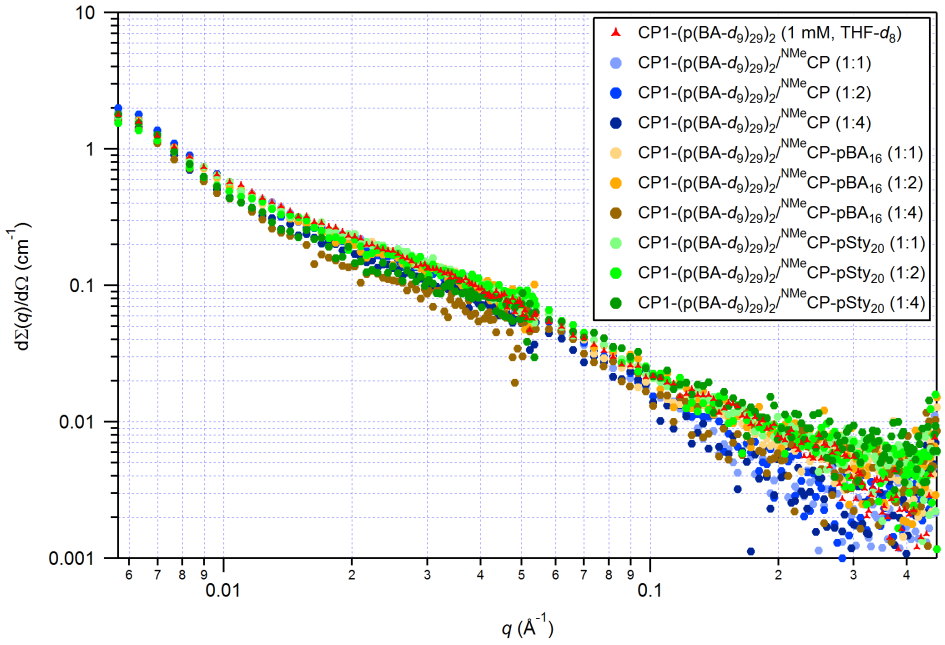

Fig. 2. SANS profiles of binary mixtures of **^{NMe}CP1**, **^{NMe}CP1-pBA$_{16}$** and **^{NMe}CP1-pSty$_{20}$** with **CP1-(p(BA-d_9)$_{29}$)$_2$** conjugates in THF-d_8 in molar ratios of 1:1, 1:2, 1:4 ([CP]:[^{NMe}CP]) keeping the ditopic concentration constant at 1 mM. Profiles shown is after subtraction of the scattering intensities of the control experiments (^{NMe}CP species and with no CP conjugates in THF-d_8).

Conclusion: In consideration of the increasing attention of synthesizing nanostructures by self-assembly, we have investigated the effect of the inclusion of monotopic species into ditopic cooperative assemblies as a simple means to manipulate the thermodynamic product. We investigated the binary mixture of (cyclic peptide)-polymer conjugates (ditopic species) and (*N*-methylated cyclic pepide)-polymer conjugates (monotopic species). We observe no change to the length of assembly up to 80 mol% monotopic species. This behaviour has been attributed to the lateral aggregation of *N*-methylated cyclic peptides, decreasing the available monotopic species to bind with ditopic species, reducing its effectiveness as chain stoppers.

1. Clark, T. D.; Buriak, J. M.; Kobayashi, K.; Isler, M. P.; McRee, D. E.; Ghadiri, M. R. *Journal of the American Chemical Society* **1998**, *120*, 8949-8962.

2. Sánchez-Quesada, J.; Isler, M. P.; Ghadiri, M. R. *Journal of the American Chemical Society* **2002**, *124*, 10004-10005

3. Koh, M. L.; Jolliffe, K. A.; Perrier, S. *Biomacromolecules* **2014**, *15*, 4002-4011