Experimental report

24/06/2017

Proposal: 9-13-668 **Council:** 4/2016

Title: Controlling the rigidity of polymeric supramolecular nanotubes

Research area: Chemistry

This proposal is a continuation of 9-10-1412

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Samples: CP-((polyethylene glycol)44)2 in D2O, Methanol d4, CDCl3, Acetone d6, THF d8 and Toluene d8
CP-((polyethylene glycol)44)3 in D2O, Methanol d4, CDCl3, Acetone d6, THF d8 and Toluene d8
CP-((polyethylene glycol)44)4 in D2O, Methanol d4, CDCl3, Acetone d6, THF d8 and Toluene d8
PEGA-co-PMA in D2O, Methanol d4, CDCl3, Acetone d6, THF d8 and Toluene d8
CP-((polyethylene glycol)44)1 in D2O, Methanol d4, CDCl3, Acetone d6, THF d8 and Toluene d8

Instrument	Requested days	Allocated days	From	To
D11	2	2	08/07/2016	10/07/2016

Abstract:

The self-assembly of (cyclic peptide)-polymer conjugates has recently been exploited to form functional nanotubes. The resulting brush-like structures are promising drug delivery vectors, form ion channels in lipid bilayers or assemble into well-defined membrane channels. These applications rely on different characteristics of the nanotubes, such as tube length, the size and type of pendant polymer chains, but also the rigidity of the supramolecular structure. Contrary to the first two parameters, the parameters influencing the rigidity are poorly anderstood. Here we propose several experiments to elucidate the influence of the solvent and the influence of the density of polymer arms on the rigidity of the self-assembled structure. And compare these results to covalently bond polymer brushes of varying polymer arms density.

Controlling the rigidity of polymeric supramolecular nanotubes – Experiment 9-13-668

This project explored the self-assembly of cyclic-peptide polymer conjugates, which interact to form nanotubes in solution. Cyclic-peptides with alternating D- and L- amino acids have been shown to self-assemble into long nanotubes via β-sheet formation.[1] This process is driven by hydrogen bond formation and hydrophobic interactions between the amino-acid backbone of the peptide. With the further conjugation of polymers, it is possible to control the physico-chemical properties of the nanotube, and increase the peptides solubility.[2, 3] However, this addition can affect the structure of the formed assemblies.

The aim of the proposed experiment had to main goals; to determine how the addition of a brush affected the self-assembly process, and to see how the number of conjugated polymer chains affected the assembly process in a range of organic solvents, including water. Fig 1 provides a summary of the structures used in these experiments.

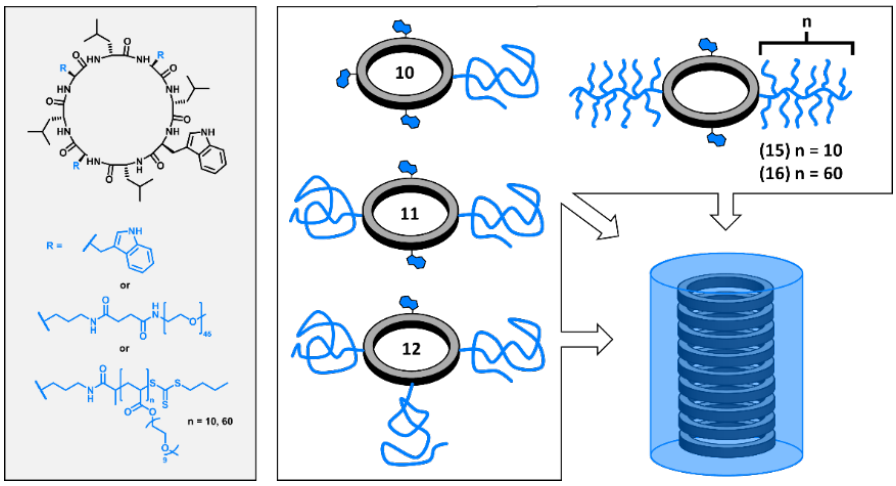
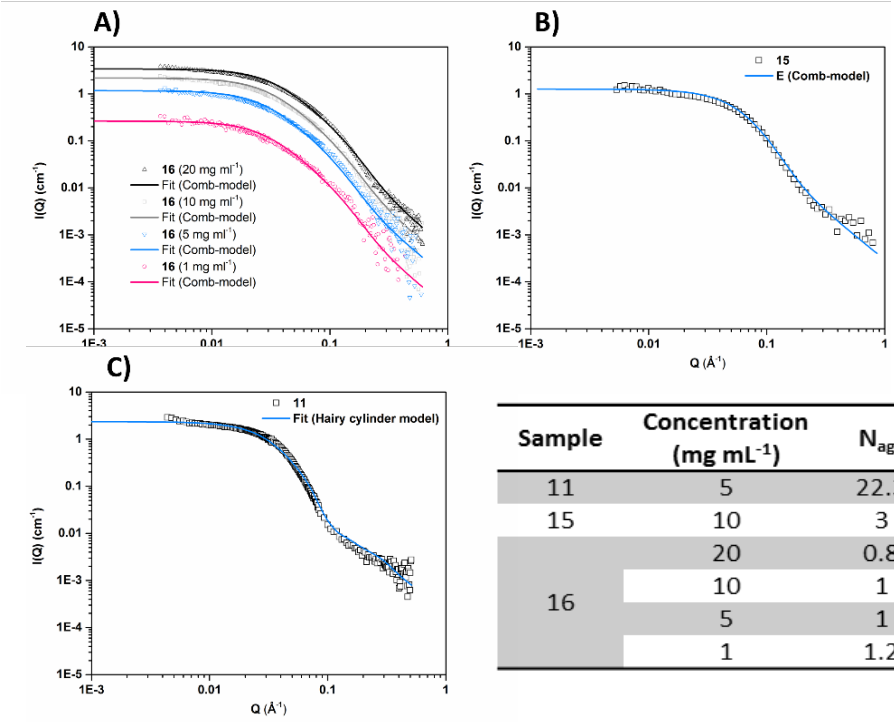


Fig 1. *Structures of the cyclic-peptide PEG conjugates. Compound 10, 11 , and 12 show cyclic peptides with 1, 2, or 3 conjugated chains, respectively. Compounds 15 and*

The first set of experiments looked how the addition of a PEG-acrylate (PEGA) brush affected the assembly of the nanotubes, when compared to a linear PEG chain. Here, 2 PEGA chains were conjugated to the peptide with varying degrees of polymerisation (Compounds **15**, and **16**), (Fig 2). Compound **16** ($CP(PEGA_{58})_2$) was measured at different concentrations, to see if any dependency existed in the data (Fig 2A). All measurements were taken in D_2O.

Compounds **15** and **16** showed the best fits to a comb model, and it was found that no interactions occurred, (N_{agg} ~1). These values were calculated based on the molecular-weight of the compounds, obtained from the relationship between the volume-fraction and SLD of the monomer, compared to the density and SLD of the solvent.[2] It has been suggested that the increased steric-hindrance around the peptide caused by the bulky brush macromolecule, is inhibiting the peptide interactions, and thus they are not able to form nanotubes. This statement is true for both the varying DP- brushes, and the different concentrations. In comparison, the linear PEG chain did assembly, and an N_{agg} of 22 was calculated.

Using the form factor for a cylindrical micelle with attached Gaussian chain (CYLINDER+Chains(RW) in the SASfit software), it was possible to determine the length of the nanotube. By dividing this value by the distance between two individual peptide units (0.47 nm),[2, 4] it is possible to determine the N_{agg} of the nanotube.



Sample	Concentration (mg mL^{-1})	N_{agg}
11	5	22.3
15	10	3
16	20	0.8
	10	1
	5	1
	1	1.2

Fig 2. *SANS data and fits of A) conjugate* ***16*** *($CP(PEGA_{58})_2$) in dependence of the concentration in water, B) conjugate* ***15*** *($CP(PEGA_{10})_2$) in water and C) conjugate* ***11*** *($CP(PEG)_2$) in water. The table provides a*

Subsequent experiments looked at how varying the number of linear PEG chains conjugated to the peptide (compounds **10**, **11**, and **12**) affected the size of the nanotubes, in a range of organic solvents. These data can be found in Fig 3, and summarised in Fig 4.

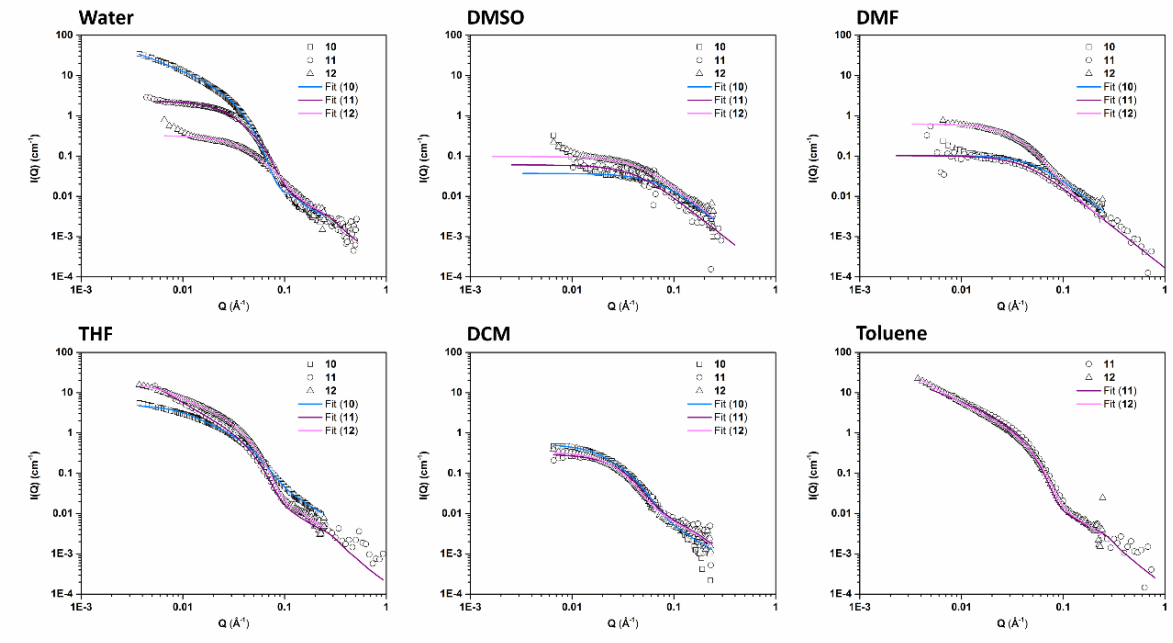


Fig 3. *Reduced scattering data for 1, 2, and 3-armed cyclic peptides (compounds* ***10****,* ***11****, and* ***12*** *respectively, with conjugated linear PEG chains (2 kDa). Solvents were chosen based on their polarity and ability to inhibit hydrogen bond interactions between peptide monomers. All data were fir to the CYLINDER+Chains(RW), apart from samples in DMSO and DMF which were modelled to a Gaussian coil.*

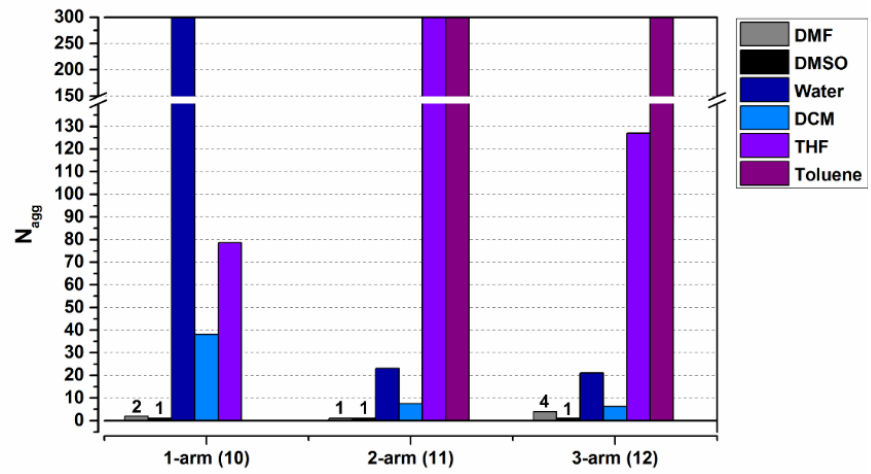


Fig 4. *Summary of the Nagg values obtained from modelling the SANS data in Fig 3. DMF and DMSO inhibited the stacking, whereas Toluene and THF formed very long tubes (N_{agg} >300).*

From these data it is clear that substantial differences exist when comparing tube-length in different solvents. Looking at the reduced scattering data (Fig 3), it can be seen that the q^{-1} intensity, indicative of cylindrical objects, does not plateau for some samples (Compound **10** in D_2O, Compound **11** and **12** in toluene, and compound **10** and **11** in THF). Therefore the actual length of the tube is outside the window of observation for SANS in these cases, and could not be determined. Based on these data as a whole, it could be possible to relate some physical parameters of the solvent and determine the ideal conditions by which a peptide could stack; and use this information to design bespoke polymers with tuneable properties to influence the stacking.

To summarise; the influence of the chain length (in the case of the brush copolymer) and the number of arms per peptide (in the case of linear PEG) on the self-assembly was studied using the provided beam-time. Various solvents were used to probe the influence of both the polarity of the solvent and its ability to interact with hydrogen bonding sites on the number of aggregation of formed nanotubes. It was found that if solvents have a high hydrogen bonding acceptor quality (DMF or DMSO), conjugates will mainly form unimers or oligomers. In solvents with moderate hydrogen bonding capacity, tubular length will more strongly rely on the ability of the solvent to overcome hydrophobic interactions between peptides. Here the properties of the polymer corona gain more importance, as the ratio between polymer and peptide influences the overall polarity of the assembly and, consequently, the ability of solvent molecules to interact with the peptide itself.

1. Ghadiri, M. R.; Granja, J. R.; Milligan, R. A.; McRee, D. E.; Khazanovich, N., Self-assembling organic nanotubes based on a cyclic peptide architecture. *Nature* **1993,** *366* (6453), 324-7.
2. Catrouillet, S.; Brendel, J. C.; Larnaudie, S.; Barlow, T.; Jolliffe, K. A.; Perrier, S., Tunable Length of Cyclic Peptide–Polymer Conjugate Self-Assemblies in Water. *ACS Macro Letters* **2016,** *5* (10), 1119-1123.
3. Chapman, R.; Warr, G. G.; Perrier, S.; Jolliffe, K. A., Water-soluble and pH-responsive polymeric nanotubes from cyclic peptide templates. *Chemistry (Weinheim an der Bergstrasse, Germany)* **2013,** *19* (6), 1955-61.
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