pH-Responsive, amphiphilic core-shell supramolecular polymer brushes from cyclic peptide - polymer conjugates

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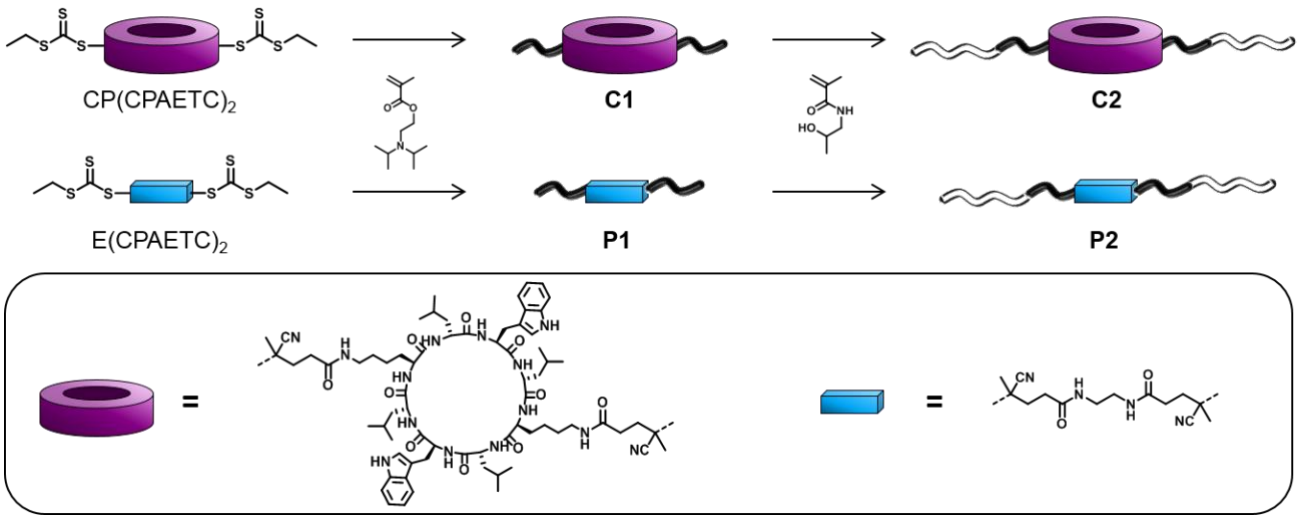
ABSTRACT: The synthesis and self-assembly of pH-responsive, amphiphilic cyclic peptide-polymer conjugates are described. The design relies on the introduction of a poly(2-(diisopropylamino)ethyl methacrylate) (pDPA) block between the cyclic peptide and a hydrophilic block. These conjugates are disassembled and protonated at low pH but assemble into core-shell nanotubes at physiological pH, as determined by a combination of titration experiments and scattering techniques.

Organic nanotubes have recently attracted considerable attention, with applications ranging from (bio)sensing to nanomedicine and electronics.[1] They can be accessed through a variety of synthetic methods but supramolecular approaches are particularly interesting thanks to their versatility and the ease of synthesis of the small building blocks.[2] A more recent development within self-assembled organic nanotubes are supramolecular polymer brushes, which are composed of a rigid supramolecular core dictating the cylindrical shape, and flexible polymer arms.[3] The driving force for the self-assembly needs to be strong enough to overcome the steric repulsion of the polymer arms and only a few systems have so far been reported to be suitable for that kind of assembly. One of the first examples relied on π-π stacking of highly unsaturated shape persistent macrocycles.[4] However in most cases strong hydrogen bonding, sometimes used together with π-π stacking,[5] is utilized to create such elongated structures, as reported for the self-assembly of bis-[3b] or tris-ureas[6] or the β-sheet stacking of peptides. A prominent example are cyclic peptides made of alternating D- and L-amino acids, which have been shown to stack in this manner,[7] and to form supramolecular polymer brushes when coupled to polymer chains.[8] The combination of these peptides with the use of reversible-deactivation radical polymerization (RDRP) techniques,[9] in particular, enabled the synthesis of a variety of well-defined polymer-peptide conjugates which assemble into the desired supramolecular polymer brushes.[8b] The use of specific monomers permits the introduction of a responsiveness,[10] which is triggered upon a change of temperature,[11] light,[12] or pH,[13] allowing the materials to change shape or size upon an external stimuli. The pH responsiveness of some polymers such as poly(acrylic acid),[14] poly(ethylene imine)[15] or poly(2-(dimethylamino)ethyl methacrylate) (pDMAEMA),[16] for example, can be used in drug delivery applications targeting the more acidic environment of tumour tissue.[17]

To demonstrate the ability of supramolecular polymer brushes to reversibly assemble upon a change in pH, our group has recently reported the synthesis of cyclic peptide-polymer nanotubes made with pDMAEMA, which can be protonated upon a decrease of pH.[18] Besides the above mentioned conjugates based on homopolymers, the self-assembly of more complex materials, which for example include a second motif for other types of self-organisation such as the formation of micelles, has barely been studied. While numerous examples for block copolymers and their self-assembly in solution are described in literature, so far only one example of conjugates of cyclic peptides and block copolymers forming nanotubes has been shown.[19] However, to our knowledge no amphiphilic system has been reported which can assemble into supramolecular nanotubes in aqueous solution. Here we describe not only the design and self-assembly of such an amphiphilic system based on blocks of the hydrophobic poly(2-(diisopropylamino)ethyl methacrylate) (pDPA), and the hydrophilic poly(2-hydroxypropyl methacrylamide) (pHPMA), but also its ability to disassemble upon a change in pH. The synthesized material is composed of a cyclic peptide core from which two pDPA-*b*-HPMA diblock copolymers arms are grown by reversible addition fragmentation chain transfer (RAFT) polymerization resulting in a core-shell system.[20] pDPA is a hydrophobic polymer which possesses a tertiary amino group and becomes hydrophilic when protonated at low pH.[21]

The self-assembly of these conjugates in aqueous solution was studied using a variety of scattering techniques

to determine whether the presence of the cyclic peptide core is sufficient to enable the assembly to be governed by the β-



Scheme 1. Synthetic route yielding the materials.

sheet formation, leading to the formation of core-shell cylindrical strutures. In addition, an appropriate triblock copolymer pHPMA-*b*-pDPA-*b*-HPMA was synthesised and analysed for comparison (Scheme 1).

The two main synthetic routes yielding peptide-polymer conjugates are grafting-to and grafting-from.[22] Various factors typically influence the choice of method, including the solubility of the different components. The cyclic peptide selected for this study is only soluble in solvents that are strong hydrogen-bond competitors, such as DMSO and DMF, in which the solubility of pDPA is limited, making the grafting-to method very challenging. For this reason, the grafting-from approach was chosen, and the CP-(pDPA)$_2$ conjugate **C1** was obtained by polymerizing DPA from the chain-transfer agent (CTA)-modified cyclic peptide CP(CPAETC)$_2$ by RAFT polymerization in a chloroform/DMSO mixture.

Table 1. Summary of polymers used in this work.

Entry	Material	$M_{n,\,th}$[a] (g.mol^{-1})	$M_{n,\,GPC}$[b] (g.mol^{-1})	$Đ$[b]
C1	CP-(pDPA$_{24}$)$_2$	11800	13900	1.27
P1	(pDPA$_{21}$)$_2$	9600	7800	1.09
C2	CP-(pDPA$_{24}$-*b*-HPMA$_{55}$)$_2$	27600	26800	1.26
P2	p(DPA$_{21}$-*b*-HPMA$_{56}$)$_2$	25600	28500	1.12

[a] Determined by ^{1}H NMR. [b] Determined by SEC using DMF (0.1% LiBr) as eluent, calibrated with pMMA standards.

The use of these conditions enabled the initial solubilisation of CP(CPAETC)$_2$ while avoiding polymerization-induced precipitation of pDPA, affording **C1** with reasonable control over the polymerization (**Error! Reference source not found.**). The control polymer **P1** that does not contain the cyclic peptide was obtained using the bifunctional CTA E(CPAETC)$_2$ in dioxane. It has been previously demonstrated that the characteristics of the polymer (degree of polymerization (DP), size of the monomer units) have a tremendous influence on the ability of the conjugates to self-assemble into nanotubes. Therefore, a DP of 25 was targeted for the DPA blocks, to provide enough protonation sites while limiting the possible steric hindrance from the bulky monomer. **C1** and **P1** were purified and used as macro-CTAs for the subsequent polymerization of the hydrophilic block. The polymerization of HPMA is best achieved in aqueous conditions in order to reach high conversions while maintaining good control over the polymerization.[23] Aqueous 1 M HCl was therefore used as the solvent in that second step, conditions under which the protonation of the DPA units of **C1** and **P1** enable the solubilisation of the macro-CTAs. A DP of 55 was targeted for the HPMA block, in order to provide sufficient hydrophilic shielding of the pDPA core. The size exclusion chromatograms of all compounds are shown in Supporting Information, Figure S1.

The pK_a of the conjugate **C2** and the polymer **P2** was then determined by potentiometric titrations of acidified solutions of the compounds using sodium hydroxide (Figure 1). Both **C2** and **P2** exhibit a distinct buffer range as can be seen on the figure, which is likely due to the fact that the charge loss resulting of the deprotonation of the DPA units triggers self-assembly of the compounds, thereby shielding the remaining charged pDPA units and making further

charge loss more difficult. This observation is indicative of a very sharp transition from free chains to assembly. The pH value at which the compounds exhibit a buffer capacity is similar for the polymer and the conjugate, however the plateau is broader for **C2**, which may indicate an additional effect of the cyclic peptide on the assembly. The degree of ionization β (which is the ratio between the amount of protonated DPA units to the total amount of DPA units in a given compound) was determined for each value of pH, and the apparent pK_a of the compounds was obtained as the pH for which β = 0.5 (see Supporting Information).[24] Both pK_a are around 7 (7.05 for **C2** and 7.10 for **P2**), which is within the expected range for pDPA polymers.

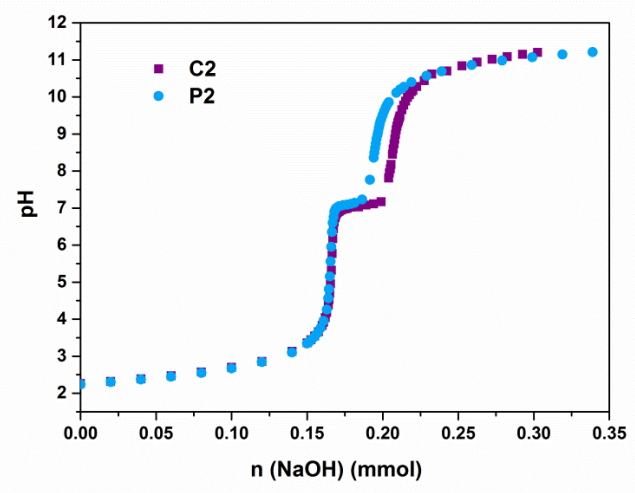


Figure 1. Potentiometric titration of **C2** (purple squares) and **P2** (blue circles).

The behaviour of the compounds in solution at pH 5.0 and 7.4 was then studied by scattering techniques. To prepare the solutions, the protonated polymers were dissolved in a given amount of water, and the same volume of 2-fold acetate or phosphate buffer was added dropwise to adjust the pH to 5.0 or 7.4, respectively. Dynamic light scattering (DLS) measurements first showed an increase in size of the compounds for both conjugate **C2** (the diameter varies from 6.5 to 14 nm) and polymer **P2** (the diameter varies from 7.7 to 11.8 nm) when increasing the pH, indicating self-assembly is taking place (Figure 2C). Interestingly, the size difference upon pH increase is higher for conjugate **C2**, which may indicate formation of bigger assemblies. However, due to the nature of the technique, which assumes spherical shapes, this result alone was not sufficient to define the assembled structures. Static light scattering (SLS) experiments were then performed, which allow for the determination of molecular weight. A study in concentration was carried out for both compounds at pH 5.0 and 7.4 (Figure 2A-B). A clear increase in size was observed for both **C2** and **P2** upon increase of pH, confirming self-assembly triggered by deprotonation of the pDPA units. At pH 5.0, where the DPA units are protonated, all concentrations result in similar scattering profiles for each compound. Moreover, a plateau is observed in both cases, allowing for direct molecular weight determination of the assemblies. Molecular weights of 79.9 ± 7.2 kg/mol and 38.7 ± 2.3 kg/mol were obtained for **C2** and **P2**, corresponding to a number of aggregation (N_{agg}) of about 3 and 1.5, respectively (see Supporting Information for details). These values confirm the fact that the electrostatic interactions are strongly hindering the self-assembly. At pH 7.4, the data sets also form an overlapping plateau for the three lowest tested concentrations (2, 1 and 0.5 mg/mL) for both the conjugate and the polymer, indicating that interactions are negligible in this range of concentrations. Molecular weights of 590 ± 42 kg/mol and 273 ± 50 kg/mol were obtained for **C2** and **P2**, corresponding to N_{agg} = 22.0 ± 1.6 and 10.0 ± 1.0, respectively. These results confirm the aggregation of conjugates **C2** into bigger structures than those formed upon assembly of **P2**. Assuming the formation of hydrogen bonds between peptides, and using the previously reported distance between two adjacent cyclic peptides,[8,29] the length of the cylinders formed by cyclic peptide-directed assembly of **C2** would be 10.4 nm. However, since SLS measurements do not provide information on the shape of the structures, complementary small angle neutron scattering (SANS) experiments were conducted (Figure 2D).[30] Both compounds were measured at each pH, and the SANS profiles at low q values clearly confirmed the size increase upon pH change, as well as the formation of bigger structures for **C2** than for **P2** at pH 7.4.

The SANS profiles at pH 7.4 were fitted using different models to determine the shape of the assemblies. For the data corresponding to polymer **P2**, the best suited model is the one of a spherical micelle, which takes into account both the spherical shape of the core and the Gaussian chains forming the corona (Figure S4).[31] Details of the fitting process can be found in Supporting Information (Table S5). Unsuccessful attempts at fitting using a Gaussian chain model (Table S3 and Figure S2) and a rigid sphere model (Table S4 and Figure S3) further demonstrate the spherical micelle configuration of **P2** at pH 7.4. These alternative models are not adapted because the former represents individual polymer chains in solution while the latter does not account for the hydrophilic blocks present on the outside of the micelle. For conjugate **C2**, both spherical micelle (Table S6 and Figure S5) and cylindrical micelle (Table S7 and Figure S6) fits were attempted. The data is best fitted with a cylindrical micelle model, demonstrating the formation of the expected core-shell like cylindrical structures. Moreover the value obtained for the height of the cylinder is in excellent agreement with the value obtained with SLS (11.3 nm by SANS *vs* 10.4 nm by SLS, N_{agg} 24 *vs* 22), hence confirming that the presence of the cyclic peptide core dictates the assembly of **C2** into nanotubes instead of simple micelles.

Fluorescence measurements were also conducted on conjugate **C2** at pH 5.0 and 7.4, and the shift of the emission peak corresponding to the tryptophan present on the cyclic peptide core clearly indicates a change in local environment polarity,[32] coherent with the self-assembly of **C2** and the formation of a hydrophobic pDPA domain at the centre of the nanotubes (Figure S7). The significant decrease of the fluorescence intensity can be attributed to self-quenching of the tryptophan groups in the assembled state.

In summary, amphiphilic, pH-responsive cyclic peptide-polymer conjugates were synthesized and their supramolecular assembly in aqueous solution was

thoroughly characterized. The combined use of DPA and HPMA afforded stabilized water soluble core-shell nanotubes at physiological pH, able to disassemble due to the protonation of the pDPA core in more acidic environments. A triblock copolymer which does not contain the cyclic peptide core was also synthesized for comparison purposes. The pK_a of both materials was determined to be around 7.0, and the structures formed by their assembly were characterized by scattering techniques at pH 7.4 and 5.0. SLS showed the distinct change in size between the different pH values in both cases, with the compounds including the cyclic peptide forming bigger

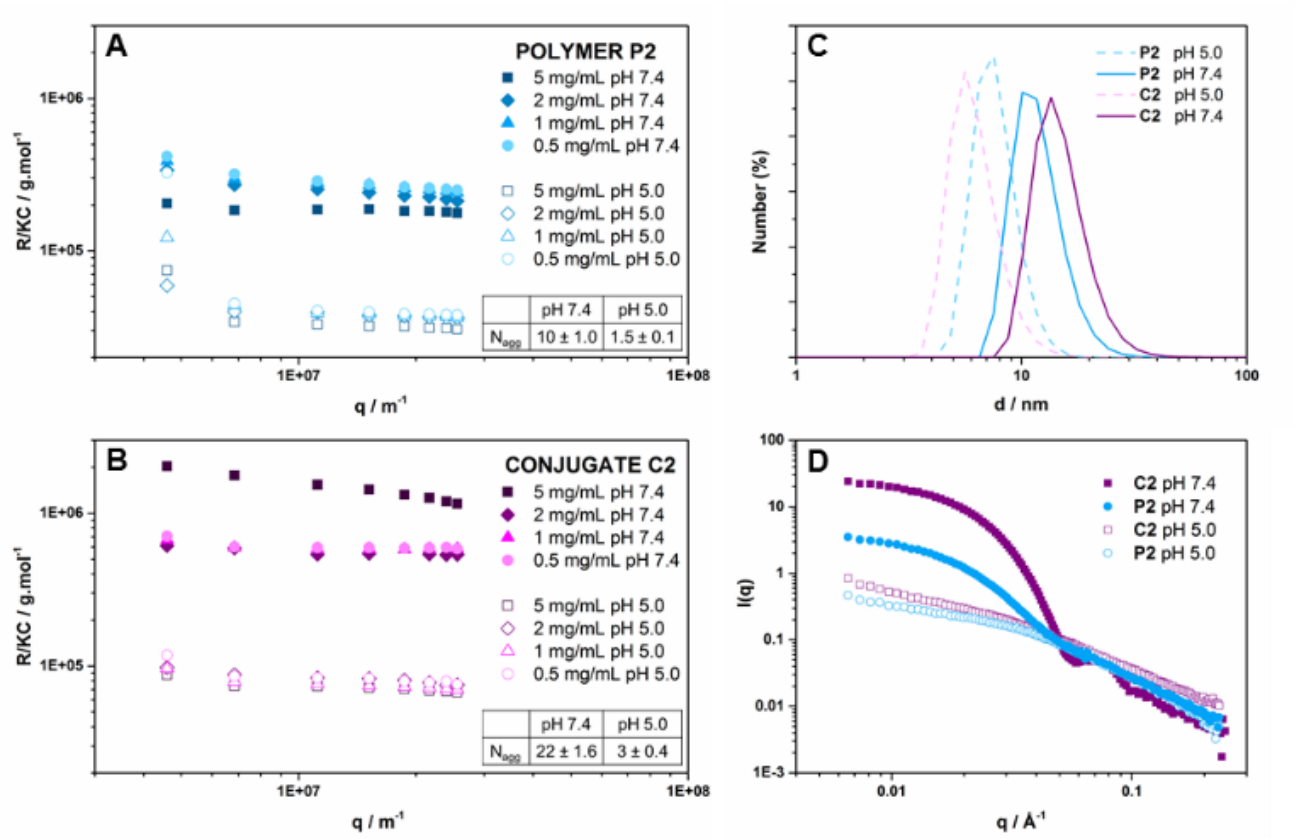


Figure 2: Characterization of self-assembly using scattering techniques. A) Static light scattering profile of polymer **P2** in solution at different concentrations and different pH values. B) Static light scattering profile of conjugate **C2** in solution at different concentrations and different pH values. C) Intensity-weighted size distribution of **P2** (blue) and **C2** (purple) at pH 5 (dotted lines) and pH 7.4 (solid lines). D) Small angle neutron scattering profiles of **P2** (blue circles) and **C2** (purple squares) in at pH 5 (empty symbols) and pH 7.4 (full symbols).

assemblies. Moreover SANS demonstrated the cylindrical shape of the peptide-containing structures in contrast to the spheres formed by the control polymer, confirming that the supramolecular stacking of the cyclic peptide core, and not the block copolymer self-assembly, governs the formation of the obtained core-shell structures. The present report introduces a water soluble, pH-responsive nanocylinder with potential applications in drug delivery. Its pK_a is indeed appropriate to trigger disassembly within endosomes following cellular uptake, thereby to facilitate endosomal escape.

ASSOCIATED CONTENT

Supporting Information. Experimental section including materials, synthesis, protocols, potentiometric titration, SANS data, and fits. This material is available free of charge via the Internet at http://pubs.acs.org.

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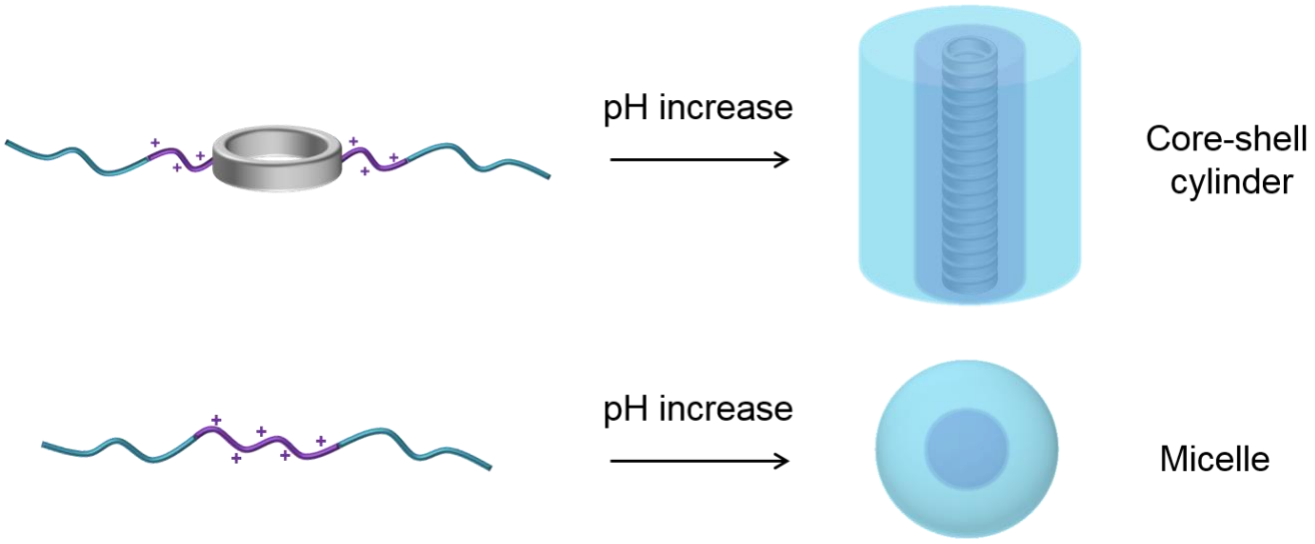
pH increase
Core-shell
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Micelle