Experimental Report

Proposal: **9-10-1385** **Council:** 4/2014

Title: Controlling Nanotube Formation in Amino Acid Amphiphiles

This proposal is a new proposal

Researh Area: Soft condensed matter

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Samples: C12KC12K

Instrument	Req. Days	All. Days	From	To
D33	2	2	12/11/2014	14/11/2014

Abstract:

In this SANS experiment we plan to study the formation process of nanotubes by amino acid amphiphiles (AAAs) as a function of the molecular constitution of the AAAs. For this purpose differently aged samples of a given AAA will be studied that cover times from mins up to 4 months. SANS shall in particular cover the size range up to 50 nm and thereby allow to deduce information regarding wall and fiber thicknesses as well as the amounts of residual micelles. This information will compared with parallel cryo-TEM experiments in which complementary information regarding the larger structures and the morphology of the self-assembled structures will be obtained. From this a comprehensive understanding of the details of the nanotube formation will be gained for the AAAs of systematically varied molecular architecture (length of alkyl chain and type of amino acid). This then shall allow to learn what are molecularly optimum compounds for nanotube formation and how one can control tube diameter and its monodispersity, which are key properties to bring nanotubes to future applications in delivery systems or as templates for metallic hollow nanowires.

Controlling Nanotube Formation in Amino Acid Amphiphiles

Experiment number:	9-10-1385
Beamline:	D33
Date of experiment:	11/12/14-11/14/14
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Introduction

The formation of well-defined nanotubes or helical fibres has been observed recently for the case of appropriately designed amino acid amphiphiles (AAAs), where the detailed structuring of the system depends on the H-bonds formed in the systems and the balance between hydrophobic and hydrophilic domains in the AAA [1,2]. Such systems are highly interesting as so far many different types of self-assembled structures are known, but especially self-assembled nanotubes are still rather scarce.

In recent work we have observed that the formation of nanotubes with such AAAs can proceed rather slowly. Of course, the formation process is very important as so far it is not fully understood, which renders the systematic formation of nanotubes with tailored dimensions rather difficult. An example of such a system is based on K:C_{12}-β_{12} (N-α-dodecyl-lysyl-aminododecyl-lysyl-amide) as AAA. Its dissolves in aqueous solution at elevated temperature in micellar form and after cooling to room temperature in a rather slow process nanotubes are formed, where this structural transition was followed by cryo-TEM [3]. This slow transformation apparently proceeds via thin fibers, then ribbons are formed, and they finally transform into the nanotubes which then are a long-time stable state. Of course the structural evolution will depend subtlety on the molecular architecture of the AAA chosen. This will be the main direction of our investigation, where we will vary this architecture in a systematic fashion and will study the structural evolutions in such systems by combined cryo-TEM and SANS experiments.

In our SANS experiments we investigated a series of AAAs, differing in length of the hydrophobic moiety (K:C_{12}-β_{12}, K:C_{10}-β_{10}) as well as the amino moiety (R:C_{12}-β_{12}), focusing on K:C_{12}-β_{12} as the main peptide. To obtain information of the nanotubulation process over time, differently aged samples of these AAAs have been analyzed in addition to freshly prepared samples, therefore obtaining information about the very early stages of nanotube formation as well as measuring at an intermediate and at a more advanced stage of the nanotubulation process. Overall samples of the ages 1, 7, 14, 16 and 17 d have been analyzed. Furthermore the prepared samples differed in concentration (1.5 mM, 3 mM and 6 mM) and pH (pH6, pH8 and pH10).

To further understand how the process of nanotube formation can be influenced by addition of amphiphiles, mixtures of K:C_{12}-β_{12} with K:C_{10}-β_{10} and R:C_{12}-β_{12} at different ratios (see table 1) have been analyzed as well as mixtures of K:C_{12}-β_{12} containing the poloxameres Pluronics® L35, F38 and P85 (0.2, 0.5 and 1 %w relative to the amount of peptide used). All samples were prepared in D_2O containing 0.05 %w of NaN_3 as a bacteriocide for enhanced sample longevity.

K:C_{12}-β_{12} + K:C_{10}-β_{10}	
c K:C_{12}-β_{12}	c K:C_{10}-β_{10}
3.0 mM	0 µM
2.995 mM	5 µM
2.975 mM	25 µM
2.9 mM	100 µM
2.7 mM	300 µM
2.0 mM	1.0 mM
0 mM	6.0 mM

K:C_{12}-β_{12} + R:C_{12}-β_{12}	
c K:C_{12}-β_{12}	c R:C_{12}-β_{12}
3.0 mM	0 µM
2.975 mM	25 µM
2.9 mM	100 µM
2.7 mM	300 µM
2.4 mM	600 µM
2.0 mM	1.0 mM
1.0 mM	2.0 mM
0 mM	3.0 mM

Tab. 1: Concentration of the individual components in mixtures prepared from K:C_{12}-β_{12} and K:C_{10}-β_{10} as well as K:C_{12}-β_{12} and R:C_{12}-β_{12}.

The SANS measurements were performed on D33 at ILL and covered a wide q-range of 0.015 – 6 nm^{-1}. The three configurations used were 1) SD=11.5m/12.5m (front and rear detector), Coll=12.8, λ=5Å; 2) SD=11.5m/12.5m, Coll=12.8, λ=5Å and 3) SD=1.2m/2m, Coll=5.3, λ=5Å.

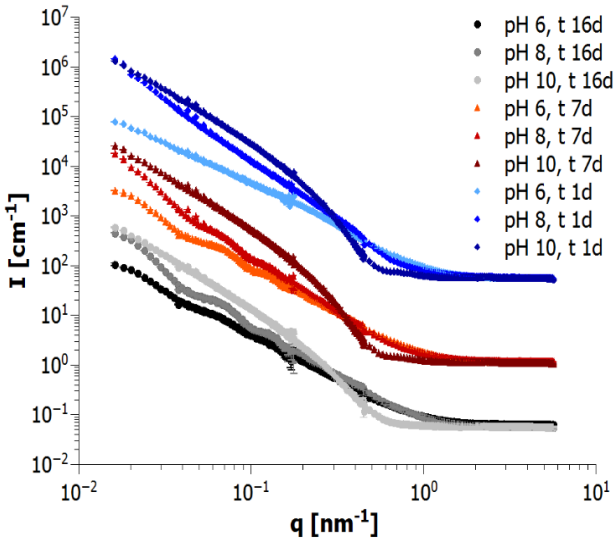

Fig. 1: SANS: Solutions of 3mM K:C_{12}-β_{12} at different pH-values and ages of the sample. Curves have been scaled to enhance clarity.

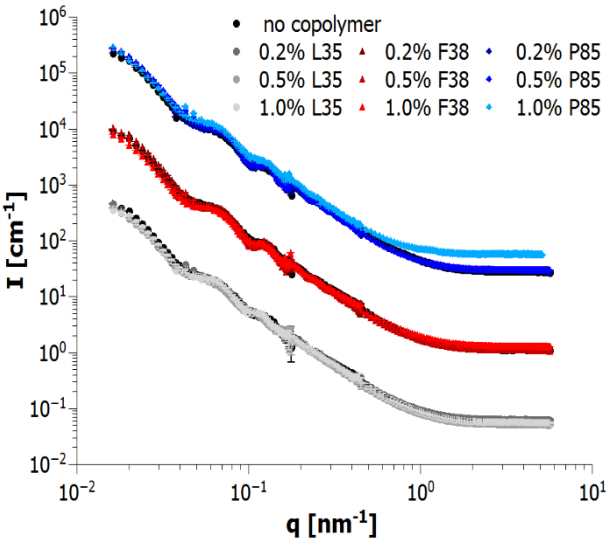

Fig. 2: SANS: Solutions of 3mM K:C_{12}-β_{12} and differently concentrated poloxameres as copolymer (Pluronics® L35, F38 and P85 respectively). All samples are at pH8, t 16d. Copolymer concentrations are relative to the amount of K:C_{12}-β_{12} per sample.

Fig. 1 shows that structures formed by K:C_{12}-β_{12} differ drastically depending on the pH of the solution. For pH6 and pH8 indications of a tubular structure can be seen; for pH8 these become more pronounced by aging. At pH10 the curves look very different and overall larger structures seem to be formed than for pH6 and pH8. Additionally, with increasing time the scattering intensity decreases for higher pH, while it increases at pH6.

Fig. 2 shows the effects of the addition of Pluronics as copolymers to K:C_{12}-β_{12}. For the case of adding L35 almost no effect on the scattering curves can be seen, apparently it has no structure directing effect. There is the indication of a slight growth of nanotube radius upon the addition of F38, but also here the changes are rather small. Similar things can be observed for the addition of P85 as well; a small increase of the nanotube radius is indicated while at the same time the polydispersity seems to increase. The addition of the Pluronic copolymers has (at best) a very

small effect on the formation of nanotubes. In future experiments it could be interesting to look at still higher Pluronic concentrations.

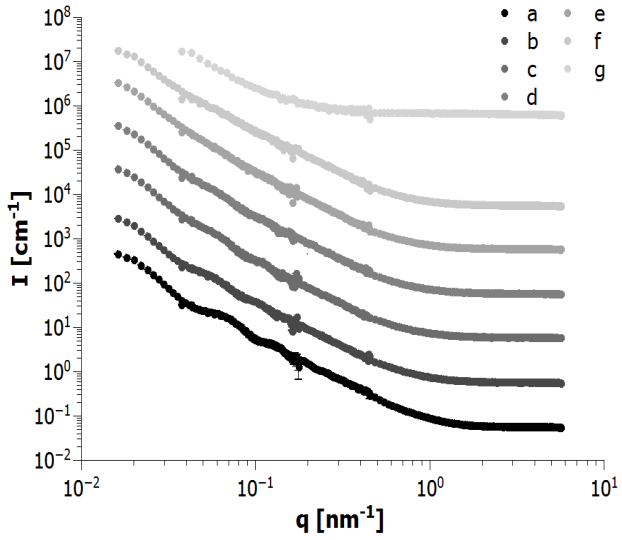

Fig. 3: SANS: Mixtures of K:C_{12}-β_{12} and K:C_{10}-β_{10} at pH8 and t 14-16d: a) 3.0 mM K:C_{12}-β_{12}, b) 2.995 mM K:C_{12}-β_{12} + 5 µM K:C_{10}-β_{10}, c) 2.975 mM K:C_{12}-β_{12} + 25 µM K:C_{10}-β_{10}, d) 2.9 mM K:C_{12}-β_{12} + 100 µM K:C_{10}-β_{10}, e) 2.7 mM K:C_{12}-β_{12} + 300 µM K:C_{10}-β_{10}, f) 2.0 mM K:C_{12}-β_{12} + 1.0 mM K:C_{10}-β_{10}, g) 6.0 mM K:C_{10}-β_{10}. Curves have been scaled to enhance clarity.

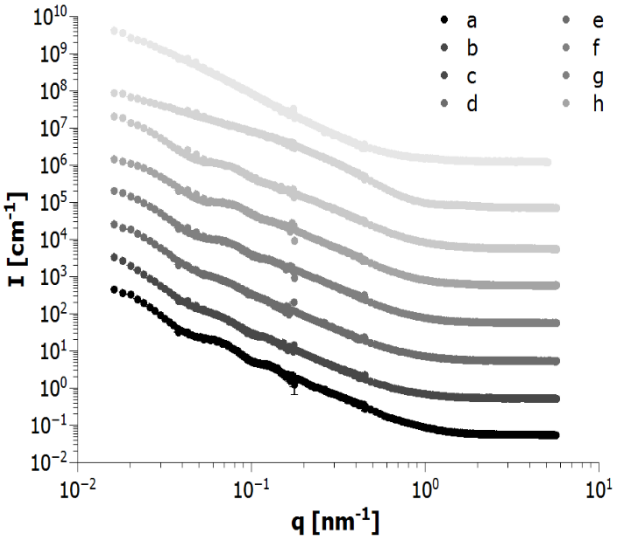

Fig. 4: SANS: Mixtures of K:C_{12}-β_{12} and R:C_{12}-β_{12} at pH8 and t 15-17d: a) 3.0 mM K:C_{12}-β_{12}, b) 2.975 mM K:C_{12}-β_{12} + 25 µM R:C_{12}-β_{12}, c) 2.9 mM K:C_{12}-β_{12} + 100 µM R:C_{12}-β_{12}, d) 2.7 mM K:C_{12}-β_{12} + 300 µM R:C_{12}-β_{12}, e) 2.4 mM K:C_{12}-β_{12} + 600 µM R:C_{12}-β_{12}, f) 2.0 mM K:C_{12}-β_{12} + 1.0 mM R:C_{12}-β_{12}, g) 3.0 mM R:C_{12}-β_{12}. Curves have been scaled to enhance clarity.

Fig. 3 and 4 show the effects of partially substituting different amounts of K:C_{12}-β_{12} for the structurally related peptides K:C_{10}-β_{10} and R:C_{12}-β_{12} respectively while keeping the overall peptide concentration constant at 3 mM.

For the addition of K:C_{10}-β_{10} (see fig. 3) it is clear that already the substitution by minute amounts has a pronounced effect on nanotube formation which becomes more pronounced with increasing the K:C_{10}-β_{10} contents. Pure solutions of K:C_{10}-β_{10}, however, have been found to scatter very little (using neutron as well as light scattering) and therefore no conclusions of the structures formed by it alone can be drawn, but these are definitely no well-defined nanotubes.

The addition of R:C_{12}-β_{12} (see fig. 4) has a rather small effect on the nanotube structure compared to K:C_{10}-β_{10}. Only after substituting substantial amounts of K:C_{12}-β_{12} for R:C_{12}-β_{12} a clear effect on the formed structures can be seen; the well pronounced features of the nanotubes become lost. This effect might be related to an influence on the nanotube formation by K:C_{12}-β_{12} or be caused by the combined signals of the structures formed by K:C_{12}-β_{12} and R:C_{12}-β_{12} alone. The pure R:C_{12}-β_{12} seems to form locally flat, lamellar structures but without showing the signature of nanotube formation.

References

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