Experimental Report

Proposal: **9-10-1390** **Council:** 4/2014

Title: Phase behaviour of di- and triblock eGFP-copolymer conjugates in aqueous media

This proposal is a new proposal

Researh Area: Soft condensed matter

Main proposer: **EDLER Karen**

Experimental Team: EL FAGUI Amani
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Local Contact: SCHWEINS Ralf

Samples: PHPMAx-b-PCLy-b-PHPMAx-eGFP conjugate in phosphate buffer/H2O/D2O
PCLx-b-PHPMAy-eGFP conjugate in phosphate buffer/H2O/D2O
PCLx-b-PHPMAy-eGFP conjugate in phosphate buffer/NaCl/H2O/D2O
PHPMAx-b-PCLy-b-PHPMAx-eGFP conjugate in phosphate buffer/CaCl2/H2O/D2O

Instrument	Req. Days	All. Days	From	To
D11	4	2	15/11/2014	17/11/2014

Abstract:

Conjugation of therapeutic proteins to polymers is well established but most methods result in multi-site and multiple conjugation, or crosslinking resulting in lack of orientation and loss of activity. Using an enzyme mediated conjugation method we have prepared well-defined protein-polymer conjugates using a model protein, green fluorescent protein (eGFP) with diblock & triblock copolymers based on hydrophilic-hydrophobic biocompatible copolymers. In this proposal we wish to determine the effects of altering the block copolymer structures on the properties of aggregates formed by these copolymers conjugates with eGFP in solution, to develop an understanding of the phase behaviour of these species.

ILL Experimental Report

Proposal number: 9-10-1390

Date of Experiment: 15-16/11/2014

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Experimental team: Amani El Fagui, Cecilia Tognoloni, Karen Edler

Local Contact: Ralf Schweins

Instrument: D11

Introduction

In order to address challenges in life sciences, self-assembly is a major tool that gives rise to a wide range of phase behaviour that is to say the formation of aggregates of various shapes, dimensions and complexity. This type of ordered materials can be obtained using surfactants (small component) or block copolymers, hyperbranched polymers, dendrimers (macromolecular species). The last decades, a new class of large species was reported. They are formed by conjugating proteins to synthetic block copolymers. The combination of the hierarchical structure and chemical functionality of proteins with the stability and highly versatile character of synthetic block copolymers may potentially lead to unique materials of both high complexity and high modularity[1].

In this study, we have gradually increased the length of the hydrophobic/hydrophilic segments of amphiphilic di- and triblock copolymers based on poly(N-(2-hydroxypropyl)methacrylamide) (PHPMA) and poly(ε-caprolactone) (PCL). These materials, obtained by reversible addition-fragmentation chain transfer (RAFT) polymerisation, were first synthesised, and then were coupled to a peptide having a diglycine ending before being conjugated to a model protein, namely, green fluorescent protein (GFP). Our ultimate goal is to find ways by which one can control the structure of the aggregates. Moreover, it is necessary to simplify our multi-component systems by selectively contrast-matching the components. In addition to varying the solvent proportion, we have used deuterium-labelled GFP. Indeed, the position of the protein and its conjugation is so far not elucidated. In order to gain structural insights this study is indispensable.

Experimental Details

The triblock copolymers spontaneously self-assemble in aqueous media (H_2O, D_2O or mixtures based on x%H_2O/y%D_2O). The conjugation reaction was carried out in mild condition [2, 3] in the presence of transpeptidase Sortase A which recognises a specific LPETGG motif (where L: leucine, P: proline, E: glutamic acid, T: threonine and G: glycine) present at the C-terminus of the hydrogen and deuterium-labeled GFP. Hydrogenated GFP was measured in D_2O and deuterated GFP in 80%H_2O/20%D_2O (to minimize the incoherent background). The protein-block copolymers conjugates were analysed in 2 solvent mixtures to observe either the signature of the protein or the block copolymer. The experiments were performed at three different samples to detector distances of 1.5, 8 and 28 m to cover a q range of 0.0021 to 0.47 $Å^{-1}$. The samples were held in rectangular quartz Hellma cells of width 1cm, thickness 1 mm and the temperature was kept constant at 25°C. The measured SANS data have been corrected and normalized to a cross-sectional unit, using the software LAMP.

Results

Very good quality data were obtained for the majority of the samples at the range of concentrations studied.

Data analysis

The scattering curves obtained indicate with no doubt that nano-objects were formed in aqueous media.

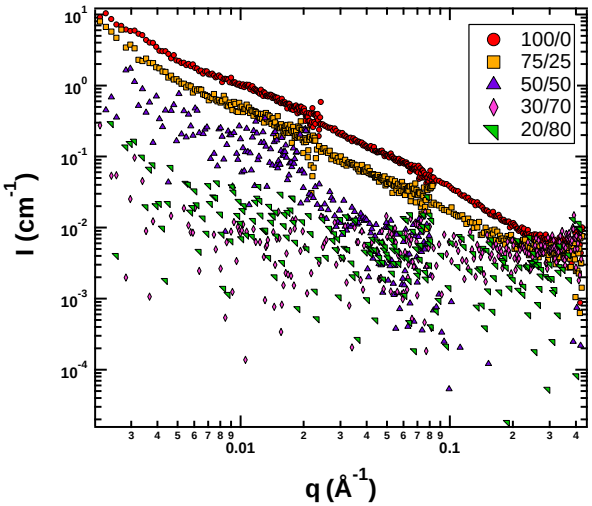

Figure 1. *SANS data for PHPMAx-b-PCLy-b-PHPMAx triblock copolymer (y=29 and x=69) for five solution contrasts [100:0, 75:25, 50:50, 30:70 and 20:80 (v/v) D_2O/H_2O]. The concentration is set at 10 mg.mL^{-1}.*

Figure 1 shows the SANS data from 1 wt% triblock copolymer. The low scattering of the 3 last solvent mixtures indicates that the blocks are almost matched out. Preliminary analysis indicated that the data are well-fitted to a core-shell sphere model.

The contrast variation technique of SANS can be used to identify the presence of the GFP. Figure 2 indicates that the scattering signatures of the block copolymers and the protein can be separated. Preliminary analysis of the scattering patterns of the protein-block copolymers conjugates using an hairy elliptical block copolymer model [4] shows that the size of the aggregates were bigger in the absence of protein suggesting a rearrangement of the structure in terms of shape making the protein-copolymer conjugates more complex aggregates.

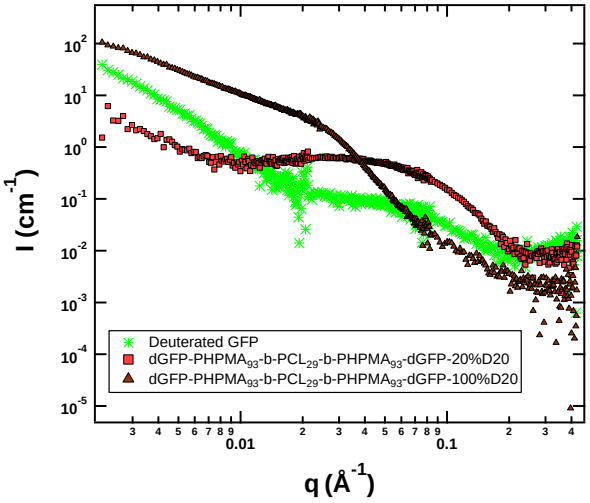

Figure 2. *SANS data for dGFP-PHPMAx-b-PCLy-b-PHPMAx-dGFP triblock copolymer (y=29 and x=93) for two solution contrasts [20:80 -copolymer matched- and 100:0 -dGFP matched- (v/v) D_2O/H_2O].*

Publications

The combination of the structural parameters that will be extricated from further data analysis of the SANS profiles with the data obtained from SAXS, cryo-TEM, NMR, IR, SDS-PAGE, and DLS will soon result in the submission of a manuscript.

References

1. Gauthier MA, Klok H-A. Peptide/ protein-polymer conjugates: synthetic strategies and design concepts. *Chemical Communications* 2008:2591-2611.
2. Chan L, Cross HF, She JK, Cavalli G, Martins HFP, Neylon C. Covalent Attachment of Proteins to Solid Supports and Surfaces via Sortase-Mediated Ligation. *PLoS ONE* 2007,**2**.
3. Piluso S, Cassell HC, Gibbons JL, Waller TE, Plant NJ, Miller AF, *et al.* Site-specific, covalent incorporation of Tus, a DNA-binding protein, on ionic-complementary self-assembling peptide hydrogels using transpeptidase Sortase A as a conjugation tool. *Soft Matter* 2013,**9**:6752-6756.
4. Pedersen JS, Hamley IW, Ryu CY, Lodge TP. Contrast Variation Small-Angle Neutron Scattering Study of the Structure of Block Copolymer Micelles in a Slightly Selective Solvent at Semidilute Concentrations. *Macromolecules* 1999,**33**:542-550.