Experimental Report

Proposal: **9-12-352** **Council:** 4/2014

Title: Morphology and Phase Diagram of Thermoresponsive Polymer-Protein Conjugates

This proposal is a new proposal

Researh Area: Soft condensed matter

Main proposer: **EDLER Karen**

Experimental Team: EDLER Karen
EL FAGUI Amani

Local Contact: SCHWEINS Ralf

Samples: HPMA-AcetylHPMA-eGFP in phosphate buffer/H2O/D2O
HPMA-AcetylHPMA in phosphate buffer/H2O/D2O
polyNIPAM-eGFP in phosphate buffer/H2O/D2O
polyNIPAM in phosphate buffer/H2O/D2O

Instrument	Req. Days	All. Days	From	To
D11	4	1	14/10/2014	15/10/2014

Abstract:

Using an enzyme mediated conjugation method we have prepared well-defined protein-polymer conjugates using a model protein, green fluorescent protein (GFP) with thermo-responsive polymers based on HPMA (N-2- hydroxypropyl methacrylamide)-Acetyl-HPMA (O-acetyl-N-2-hydroxypropyl methacrylamide) and a commercially available PNIPAM-NH2. In this proposal we wish to study the self-assembly of these temperature responsive polymer-protein conjugates. Such investigations are required to understand the structural properties of these materials as the molecular weight changes and how they respond to the polymers transition to a collapsed state at higher temperatures. The phase diagram of eGFP-copolymer conjugates will be mapped out with respect to copolymer composition and concentration effects as well as temperature and water content.

ILL Experimental Report

Proposal number: 9-12-352

Date of Experiment: 14/10/2014

Title: *Morphology and Phase Diagram of Thermoresponsive Polymer-Protein Conjugates*

Experimental team: Amani El Fagui, Karen Edler

Local Contact: Ralf Schweins

Instrument: D11

Introduction

Our project focuses on the development of protein-polymer conjugates as a supramolecular targeted therapy for atherosclerotic cardiovascular diseases. Polymeric supramolecular assemblies such as micelles, nanoparticles and vesicles are more stable than small molecule counterparts, and their designs and morphologies can be optimised by e.g the design of polymer-protein conjugates.

The properties of the conjugates strongly depend on the conjugation strategy as well as the type of the macromolecular species chosen. By using a selective and specific methodology, it is possible to attach the polymer to the protein at one unique site. The materials obtained have therefore the same construction where the protein is found in only one orientation to exert its function [1] (eg. targeting, diagnostic and affinity separation).

In this study, commercial hydrophilic polymers viz amino-terminated poly(N-isopropylacrylamide) (PNIPAM) were used. *This is our first measuring campaign using Small Angle Neutron Scattering to study a site-specific conjugation methodology applied to commercial thermoresponsive polymers.*

Experimental Details

The conjugation reaction selected [2, 3] requires an entity bearing diglycine moieties and the presence of a C-terminus LPETGG sequence in the protein (where L: leucine, P: proline, E: glutamic acid, T: threonine and G: glycine) which will be recognised by an enzyme named transpeptidase Sortase A. In this study, GFP, a green fluorescent protein, was chosen as a model. The solutions for SANS measurements were prepared as a function of the molecular weight of the commercial PNIPAM-NH_2 and the concentration of the protein. The first step was to graft a diglycine tag to the polymers and then attach the protein in presence of the enzyme. The solutions, prepared in deuterated water to have the strongest contrast, were previously fully characterized (NMR, IR, SDS-PAGE, and DLS) at the University of Bath. The experiments were performed on D11 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 1 cm, 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 all samples at the range of concentrations studied. All the curves enable us to extract structural parameters. However, due to a lack of time the samples were not measured at 37°C as planned.

Data analysis

The initial analysis of the obtained spectra allowed asserting that we have successfully prepared protein-polymer conjugates. Figure 1A reveals that at intermediate q, the slope equals ~1 which indicates the presence of rigid rods in the solutions. This signature can be made clear by using the so-called Casassa-Holtzer plot (qI(q) against q).

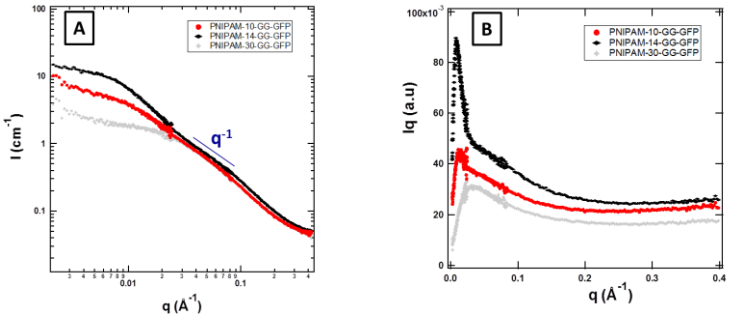

Figure 1. *Representative Small Angle Neutron Scattering data of solutions based on GFP grafted to a series of PNIPAM with different molecular weight bearing a diglycine motif.*
Scattered intensity I(q) versus wavevector q in a log-log representation ***(A)***
and their corresponding Casassa-Holtzer plot ***(B)****.*
-Some curves are offset for clarity-

The position of the maximum enables us to estimate the Radius of Gyration of the nano-objects in solution and their number of Kuhn lengths which depends on the height of the peak. Unexpectedly, a higher peak height is obtained for the intermediate PNIPAM Mw (14kDa) studied.

So far, we have considered scattering behaviour only in terms of asymptotic expressions (Guinier, Porod, Casassa-Holtzer…). In the next phase of the data analysis, the SANS NIST Package will be used to obtain a complete quantitative characterisation of the scattered intensity over the entire q range.

Publications

These SANS results have been supplemented by Small Angle X-ray Scattering measurements. Therefore the combination of the structural parameters obtained from data analysis of the SANS profiles with the data obtained from SAXS, NMR, IR, SDS-PAGE, and DLS will soon result in the submission of a manuscript.

References

1. Hoffman AS, Stayton PS. Bioconjugates of smart polymers and proteins: Synthesis and applications. Macromolecular Symposia 2004,207:139-151.
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.