Experimental report

19/03/2024

Proposal: 9-10-1761 **Council:** 10/2022

Title: Microstructure of poly-L-lysine (PLL) single chain nanoparticles: therole of the precursor conformation

Research area: Soft condensed matter

This proposal is a new proposal

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Samples: poly-L-Lysine/NaCl/D2O

Instrument	Requested days	Allocated days	From	To
D22	2	1	03/07/2023	04/07/2023

Abstract:

Single chain nanoparticles (SCNPs) are unimolecular polymer chains folded or collapsed via intra-molecular cross-linking under high dilution, leading to sparse conformations and a topological polydispersity similar to that of intrinsically disordered proteins. Due to their small size, softness, and internal compartmentalization they have applications in catalysis, sensing and drug delivery. To extend SCNPs to polypeptides, it is important to understand the role of the chain conformation of the precursor on the resulting SCNP morphology. This proposal aims to probe the conformation of SCNPs based on the polyelectrolyte poly-L-lysine (PLL) using SANS. We hypothesize that the polymer conformation has an important role on the balance between inter and intramolecular cross-linking as well as on the internal morphology of the SCNPs.

Microstructure of poly-L-lysine (PLL) single chain nanoparticles: the role of the precursor conformation.

Single chain nanoparticles (SCNPs) are unimolecular polymer chains folded or collapsed via intra-molecular cross-linking under high dilution, leading to sparse conformations and a topological polydispersity similar to that of intrinsically disordered proteins (IDPs). Due to their small size, softness, and internal compartmentalization they have applications in catalysis, sensing and drug delivery. The size reduction caused by the chain collapse is typically deduced from size exclusion chromatography and dynamic light scattering. However, changes in the polymer conformation, i.e, the internal degree of compaction, are best probed with small angle scattering.
Recently, there has been great interest in expanding this technology to biodegradable and biocompatible polymers. Thus, we adopted a strategy to internally cross-link poly- L- lysine (PLL) using suberic acid bis (3-sulfo-N-hydroxysuccinimide ester) sodium salt (BS^3) that reacts with lysine moieties in a polypeptide. PLL-SCNPs have enormous potential due to their biocompatibility and low immunogenicity, therefore it is important to understand the role of the precursor chain conformation on the resulting SCNP morphology. This proposal aimed to probe the conformation of SCNPs based on poly-L-lysine (PLL) using SANS.

To study the effect of the PLL chain conformation on the SCNP morphology, we investigated the structure of PLL linear chain (Mw~ 150kDa) and SCNPs at approximately 3 mg/mL with 3 NaCl concentrations (0.5 and 1 and 2M) at 2 neutral to basic pH conditions: pH = 7.4 and 9. The SCNPs were synthesized with BS^3 at 10 and 30 mol% added with respect to the total monomer concentration and then filtered to separate from large aggregates arising from inter-molecular crosslinking events. The SANS measurements were performed on the D22 instrument. Two configurations were employed at 2 sample-to-detector distances: 1.4 m and 17.6 m and wavelength of 6Å to cover a Q-range from 0.03 to 7 nm^{-1}. Samples were placed in 2 mm thick quartz banjo-type cuvettes in a multiple-slot sample changer with controlled temperature of 25 °C and measured for 30 minutes with the neutron beam aperture of 12 mm. The scattering data were normalized for the empty beam, empty cell, sample transmission and background corrected (D_2O). Data reduction was performed using Grasp- a MATLAB script application for the analysis and reduction.

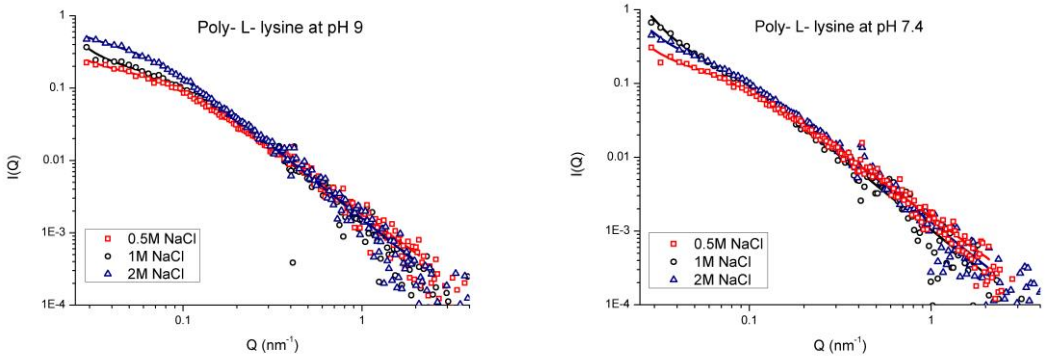

Figure 1. SANS data of 3 mg/mL poly- L- lysine at 2 different pHs: 9 (left figure) and 7.4 (right figure) at different salt concentrations. Solid lines correspond to fits using a generalized Gaussian coil and taking into account the low-q contribution.

The initial conformation of the PLL chain in different solvent conditions were systematically investigated: salt concentrations were varied from 0.5M to 2M at 2 different pHs (7.4 and

9) (Figure 1). At basic pH (pH=9), the ionic strength has a clear effect on the polymer structure. The addition of salt concentration leads to the gradual increase of the forward scattering. In the case of neutral pH (pH=7.4), the change is not apparent: at different salt concentrations there is less change in the scattering curves.
Then, we studied the effect of cross-linking, i.e., SCNP formation, on the chain conformation. Figure 2 shows as an example the scattering profiles for crosslinked PLL at 3 mg/mL in 2 different pHs (7.4 and 9) at 2M NaCl as a function of the added cross-linker BS^3 concentration. The scattering curves of the crosslinked PLL have a conformation close to that of a random coil. In fact, preliminary data analysis shows that the model for a generalized Gaussian coil form factor describes well the data of the PLL as well as the PLL-SCNPs.

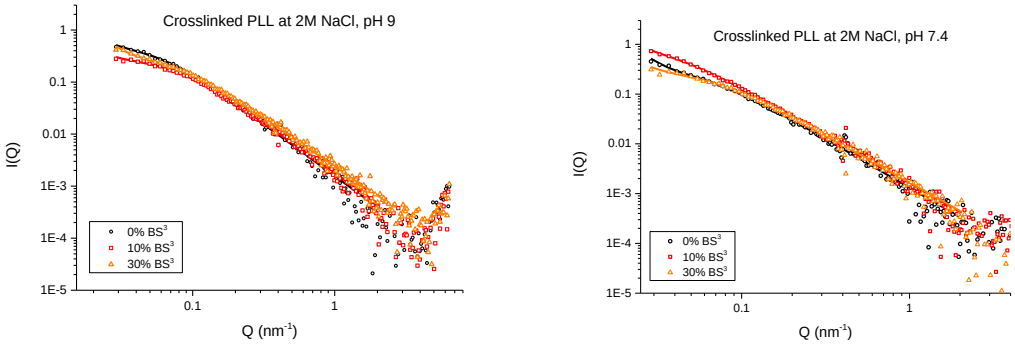

Figure 2. SANS data of crosslinked 1mg/mL PLL in 2M NaCl at 2 different pHs: pH 9 (left figure) and pH 7.4 (right figure) at different crosslinker concentrations. Lines correspond to the model fit analysis with a generalized Gaussian coil form factor and take into account the low-q upturn.

At pH 9, with the addition of crosslinker the chain is probed to be collapsed in terms of gyration radius (R_g) with a sparse conformation due to the increase in ν. However, it is not the case for PLL at pH 7.4. Further analysis will help elucidate the different mechanisms of SCNPs formation.