Experimental report

06/09/2024

Proposal: **9-11-2209** **Council:** 4/2024

Title: Crowded Solutions of Irreversible Single-Chain Nano-Particles: Transition to Crumpled Globular Conformation at High Concentrations

Research area: Soft condensed matter

This proposal is a continuation of 9-11-2137

Main proposer: **Maria Aranzazu ARBE MENDEZ**

Experimental team:
Maria Aranzazu ARBE MENDEZ
Maria Isabel ASENJO SANZ
Ainara RUIZ

Local contacts: Lionel PORCAR

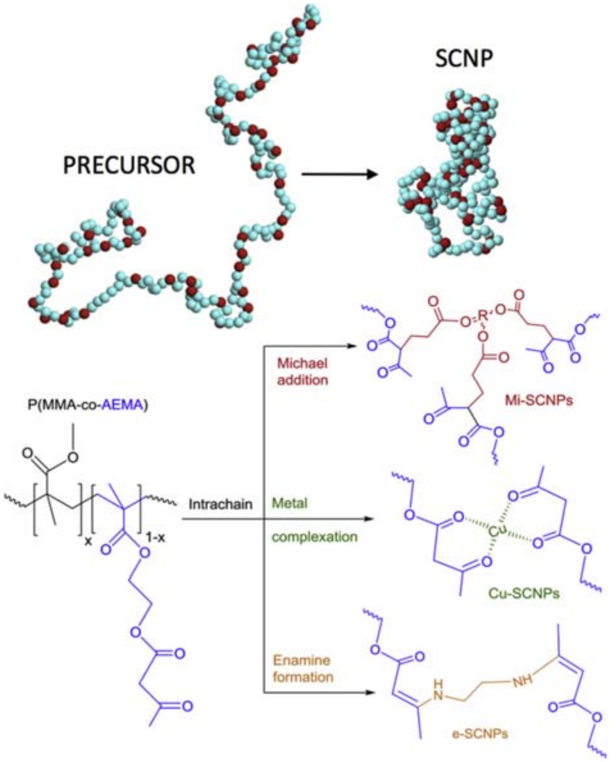
Samples:
hPrecursors and dPrecursors in solvent
Background solvents (h/d DMF)
hSCNPs and dSCNPs in solvent

Instrument	Requested days	Allocated days	From	To
D22	2	1	16/05/2024	17/05/2024

Abstract:

Single-chain nanoparticles (SCNPs) are promising nano-objects in the nano-technology field and can be considered as good model systems for intrinsically disordered proteins. Characterizing their behavior in crowding environments is thus a highly interesting problem. In this project we aim to characterize the intramolecular conformations of SNCPs in solution, exploring a broad range of concentrations from the dilute to the concentrated regime and melt, and to establish differences with the behaviour for crowded solutions of reversible and irreversible SCNPs. We have already demostrated the insensitivity of chain conformation upon crowding for the reversible case, as predicted by our simulations. Now we want to prove the transition to crumpled globular conformation in irreversible SCNPs, as well as the transition to random coil in the linear precursor chains. The involved experiments are a clear showcase of the unique capabilities offered by high-concentration label techniques with neutron scattering.

SANS experiments were carried out on solutions (in dimethyl formamide, DMF) of irreversible single-chain nano-particles (SCNPs) obtained through Michael addition (see scheme below), using linear copolymers of methyl methacrylate (MMA) and (2-acetoacetoxy)ethyl methacrylate (AEMA) as precursors. Solutions were filling Hellma cuvettes of 1mm thickness. A wavelength of 6Å was used, with two collimations set up (17 and 4m). To exploit labelling, we used protonated macromolecules (M_w=237kDa, PDI=1.27) and deuterated macromolecules (M_w=249.9kDa, PDI=1.37). The starting precursors were the same as those used for synthesizing the reversible SCNPs investigated in the previous experiment, i. e., macromolecules where the MMA monomer is deuterated and the AEMA monomer is protonated. Since this is the minority component (29%), the SLD is still much higher than that of the fully protonated chains. The molecular weight of protonated and deuterated chains was such that they contain a very similar number of monomers, as required in the experiments we performed.



Scheme. Upper part: MD-simulation snapshot of a linear precursor chain and a SCNP obtained through internal cross-linking processes. Red beads represent the reactive groups. The lower part shows three particular synthesis routes that can be applied to the precursor chains used in this experiment.

Our interest was to access the form factor of the macromolecules in solutions with increasing concentration. This requires high-concentration label methods. The experiments thus consisted of mixing protonated and deuterated polymers (with a volume fraction ϕ of deuterated stuff) with total polymer concentration c in a solvent of SLD ρ_s. We thus had to determine the ZAC condition

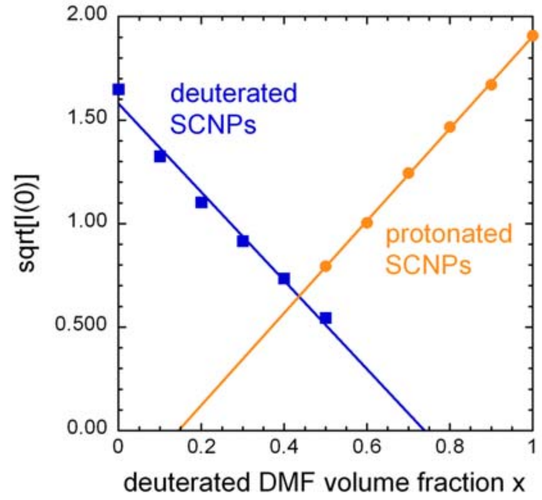
$$\phi \rho_d + (1-\phi) \rho_h - \rho_s = 0$$

where ρ_d (ρ_h) is the SLD of the deuterated (protonated) chains. We chose ϕ =0.5 to maximize the intensity of the intramolecular contribution to the scattering, weighted by $\phi (1-\phi) (\rho_h - \rho_s)^2 nV^2$ (n: number of chains per unit volume; V: volume of the macromolecule). Then, ZAC condition requires

$$\rho_d - \rho_s = \rho_s - \rho_h$$

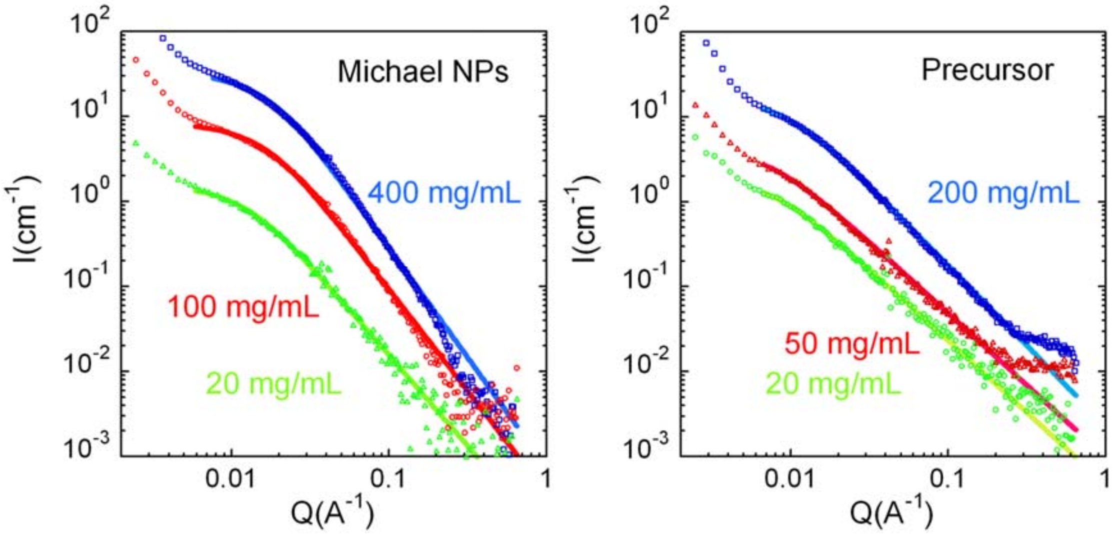
This was experimentally determined by measuring solutions of either deuterated or protonated SCNPs (at 10mg/mL) in solvents with varying SLD, i. e., with different volume fraction x of deuterated DMF.

Since the forward intensity I(Q->0)=($\rho_{d(h)}$ - ρ_s)^{2}nV^2, its sqrt is proportional to $\Delta\rho$=abs($\rho_{d(h)}$ - ρ_s). The ZAC condition was determined from these values, and was found to be x=0.43 (see Figure below).



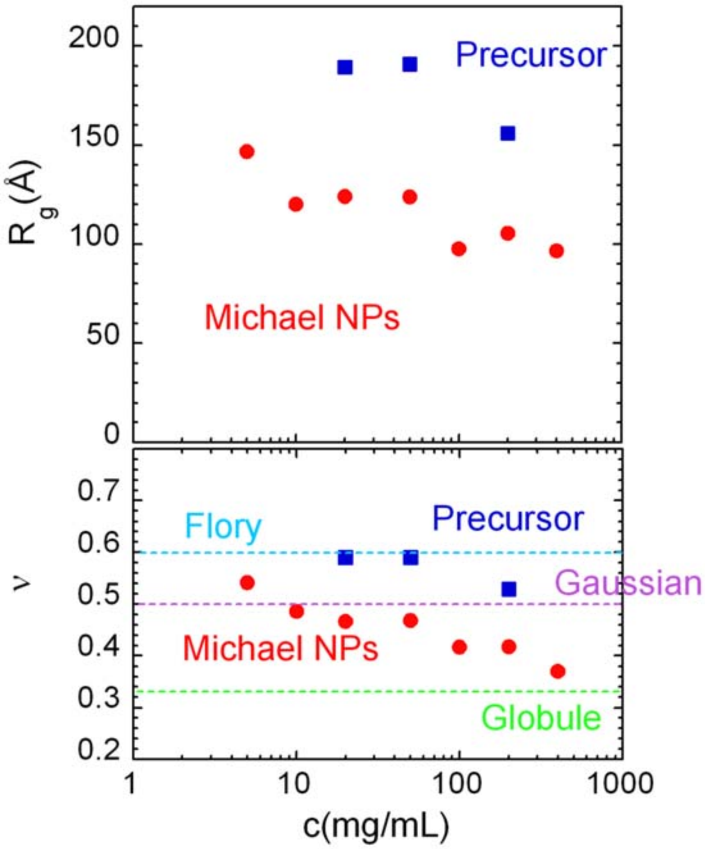
Samples in ZAC were prepared satisfying this condition. Since filling cuvettes with samples at high concentrations is very difficult (they are extremely viscous), pre-disolved samples with a first guess estimation of the solvent composition had been prepared in the home laboratory, and the final composition was tuned after this determination. The experiment was limited to a maximum concentration value of 400mg/mL.

Some representative results of the SANS experiments on the irreversible SCNPs in ZAC are shown in the Figure below (left).



Analogous solutions of the precursor chains were also investigated, in the same ZAC condition. The results are shown on the right panel figure of the figure above. The slopes in the fractal regime are obviously more pronounced in the SCNPs results, reflecting more compact coformations.

From the fits of generalized Gaussian coils to the results, we obtained the size (radius of gyration, R_g) and scaling exponent ν as function of concentration. They are represented in the figure below, comparing the two systems.



Confirming our expectations, with increasing macromolecular concentration the chain dimensions decrease in both cases. The collapse of the chains is clearly revealed by the decrease of the scaling exponent. For the precursor chains, this parameter shows the value of the Flory exponent corresponding to a self-avoiding walk at low concentrations (0.59) and at high concentrations tends to the value of ½ typical for a random coil (Gaussian chain) expected for polymers in bulk conditions. On the contrary, the scaling exponent of the SCNPs in dilute conditions is, due to the intramolecular cross-links, close to that of a Gaussian chain and tends to approach that of a crumpled globule in the high-concentration limit, as predicted by our simulations. Thus, the behaviour is markedly different with respect to that observed for the reversible SCNPs investigated in our previous experiment.