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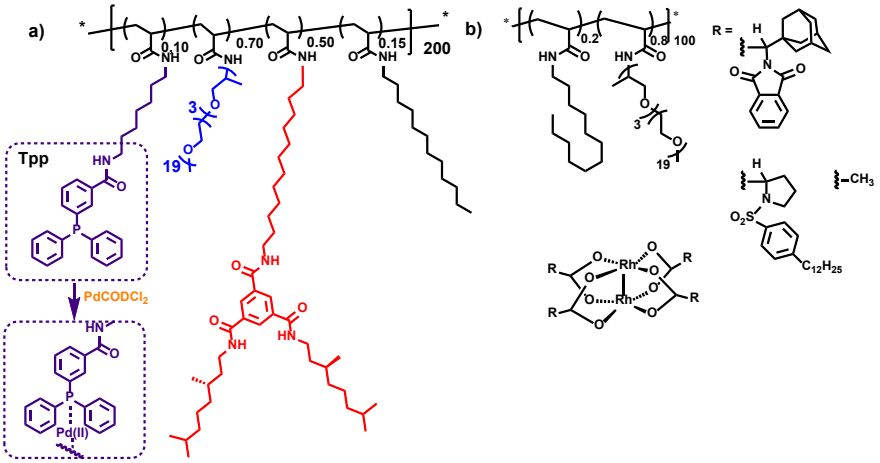
Application for beam time at ESRF on BM29 – Experimental Method

Proposal Summary (should state the aims and scientific basis of the proposal) :

Single chain polymeric nanoparticles (SCPNs) formed from amphiphilic heterograft polymers are versatile platforms in biomedicine as drug delivery vehicles, sensors or for imaging. We are developing SCPNs as bio-orthogonal catalysts for catalysis in vitro en-route to in situ drug synthesis for cancer therapies and reduced side effects. We developed transition metal loaded nanometre-sized polymeric nanoparticles loaded with Pd, Cu, or Rh-based catalysts, for specific reactions in water and bio-media. We are currently studying the structure-activity relationships both at ensemble and single molecule level, on amphiphilic polymers with variations in hydrophobic, hydrophilic and supramolecular grafts content. Therefore, it is of great interest to study the size, shape, and compactness of these catalytic nanoparticles in order to understand the influence of their microstructure on the catalytic activity.

Scientific background :

We developed SCPNs (DP = 200) loaded with either covalent Pd, Cu catalysts or encapsulated Rh catalysts. All three systems are catalytically active in water and promising for in vitro applications. An general overview of the systems and previous characterization is shown in Scheme 1 and Figure 1. DLS measurements revealed a mostly monomodal scattering peak of particles with a hydrodynamic radius of 4-5 nm for all systems, with a small amount of much larger aggregates present (r_H = 50 nm). SAXS measurements were performed on an in-house SAXSlab Ganesha system at low concentrations to minimize aggregation, to determine the size and shape of the particles. Particle scattering was observed, but even with long measurement times (7h), the scattering statistics were too poor to accurately determine the size and shape of our particles by fitting the form factor. Additionally, we would like to use SEC-SAXS on model systems to remove small amounts of aggregates (r_H = 50 nm) to improve the quality of the scattering curves, especially to reach plateaus in the low q region, as long as sufficient polymer concentrations can be achieved in the elution volume.



Scheme 1: Two catalytic systems based on SCPNs a) ***P1*** *loaded with Pd(II) b)* ***P2*** *loaded with Rh(II)*

Experimental technique(s), required set-up(s), measurement strategy, sample details :

Through SAXS in H2O we would like to accurately determine the shape, size, and compactness of the polymer systems. SAXS has been used successfully for polymers with high scattering densities in the past.[1,2] From our previous experiments, we know that a beamline with high intensity and brilliance is essential to get good statistics. We expect that the inclusion of metal catalysts in SCPNs will result in high scattering intensities, and hence enable the accurate determination of the particle's structure (guideline rH = 4-5 nm). Rg and MW will be determined through the Guinier analysis, whereas the particle shape will be determined via form factor modelling of the scattering curves. The shape factor ρ can then be determined

by combining Rg from SAXS with RH from DLS. Mw as derived from SAXS reveals the number of polymer chains per particle by dividing with the theoretical molecular weight of a single polymer chain. This can be done with sufficient accuracy as long as the scattering curve reaches a plateau at low q. Through these experiments, the particle structure enables investigation of the structure-activity relationships of catalytic SCPNs.

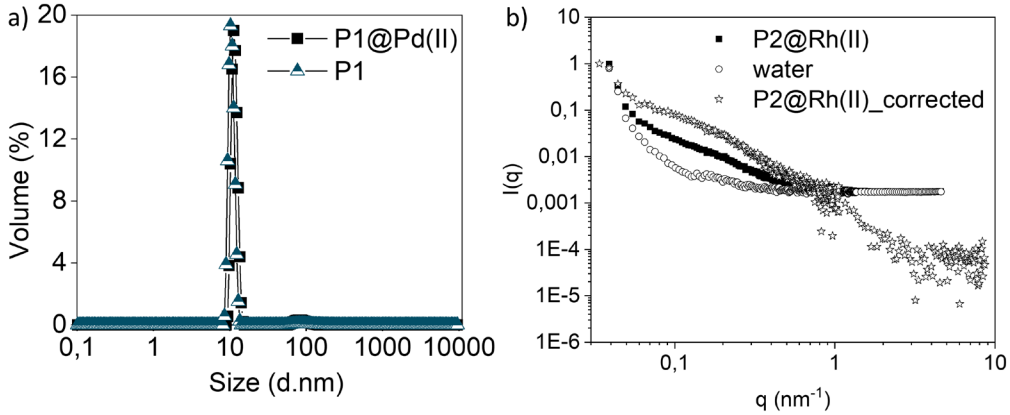


Figure 1: DLS in water. (SCPNs with and without Pd catalyst (left). SAXS measurements on a SAXSlab-Ganesha (right). P2@Rh(II)_corrected is the form factor. cpol = 1 mg mL^{-1}.

Beamline(s) and beam time requested with justification :

For the current study, we prepared samples with 3 catalysts, 4 polymers per catalysts, which we intend to measure both before and after complexation, at 5 different concentrations in H2O: 0.25, 0.5, 1, 2, and 5 mg mL^{-1} at room temperature, for a total of 3 x 4 x 2 x 5 = 140 SAXS samples. From the DLS data, we observed that the samples contain 1-2 vol% of much larger (irreversible) aggregates (r_H = 50 nm). A concentration series is therefore important to investigate the aggregation tendencies and reach a low q plateau to determine MW. We stress here the importance of measuring on a low background beamline such as BM29 due to lower contrast compared to e.g. enzymes of similar size. Per sample, we expect to require 5min of beamtime for good statistics (measuring multiple separate frames to minimize radiation damage), for a total time of 12h. Additionally, we would like to use SEC-SAXS to remove the aggregates even at higher concentrations to improve the sample quality. For this, we aim to measure 12 samples, at minimum 30min per sample. Including additional time for initial setup, absolute calibration, measurement of the direct beam, capillary, transmission runs, etc. we kindly ask for 24h (3 sessions of 8h) beamtime on BM29.

Results expected and their significance in the respective field of research :

The current system is intended to be used in a bio-orthogonal setting. For such applications, the control over the size and shape of the nanoparticles is of utmost importance. The way in which the particles are folded around the catalysts will ultimately determine the interactions between the polymeric nanoparticles and the surrounding competitive biomolecules. Therefore, with the requested SAXS time on BM29, we intend to determine the size, shape, and compactness of the polymeric systems for the different metal-ligand complexes before and after complexation. The observed structure-property relationships will reveal which systems are likely candidates for eventual application as bio-orthogonal catalysts in living cells.

References

1. Ter Huurne, G. M., Gillissen, M. A. J., Palmans, A. R. A., Voets, I. K. & Meijer, E. W. The coil-to-globule transition of single-chain polymeric nanoparticles with a chiral internal secondary structure. *Macromolecules* **48**, 3949–3956 (2015).
2. Ter Huurne, G. M. *et al.* The effect of dendritic pendants on the folding of amphiphilic copolymers via supramolecular interactions. *J. Polym. Sci. Part A Polym. Chem.* **57**, 411–421 (2019).