Understanding Protein Corona Formation under Shear Flow Conditions

Karim Daramy[1], Yvonne Perrie[1], Zahra Rattray[1]

1. Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, Glasgow.

E-mail: karim.daramy@strath.ac.uk

Introduction:

Nanoparticles are small colloidal particles in the 1-100 nm size range. Polymeric nanoparticles are routinely explored for the development of novel drug delivery systems due to their unique and customizable physical and chemical characteristics.

Upon introduction to protein-containing medium, nanoparticles will spontaneously adsorb proteins onto their surface and form what is known as the 'protein corona' (**figure 1**) The protein corona leads to changes in the physical and chemical parameters of nanoparticles, which subsequently alters their biological fate (cellular uptake, biodistribution). With most nanoparticles intended for intravenous administration, it is crucial to understand the impact of biological shear flow conditions on protein corona formation and how the protein corona influences their colloidal stability.

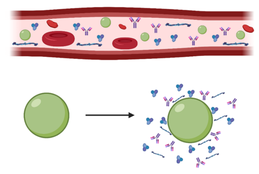


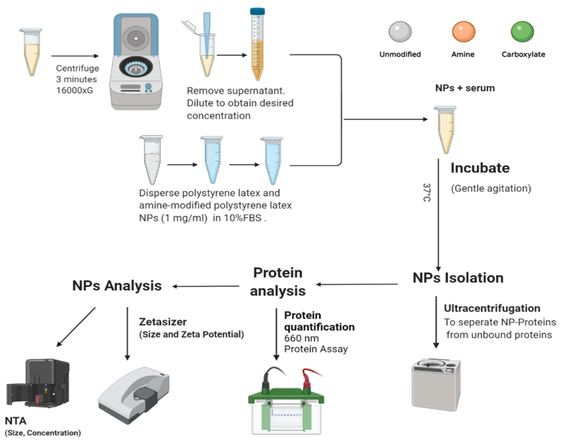
Figure 1. Schematic showing protein corona formation on nanoparticle surface following injection.

Aims & Objectives:

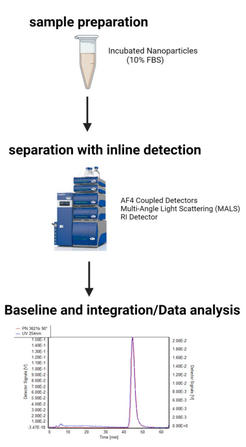
To characterise the changes in particle parameters following incubation under various shear flow conditions mimicking physiological conditions. We will use a range of particle isolation and analytical techniques to measure the impact of sample preparation conditions on nanoparticle parameters using model polystyrene latex nanoparticles.

Method:

Pipeline for comparison between different surface functionalization



In situ asymmetric Field Flow Fractionation (FFF) Pipeline



Results:

1) The impact of incubation time on polystyrene latex nanoparticle parameters

Table 1. Nanoparticle parameters measured by NTA and DLS before (baseline, 0 hours) and after incubation with Phosphate-buffered saline (PBS) containing 10% v/v fetal bovine serum (FBS) for 2 and 24 hours.

Particle	Size, NTA (nm)	Z-average (nm)	PDI	Zeta Potential (mV)
Unmodified				
0 hr	105.0 ± 0.2	118.0 ± 0.5	0.04 ± 0.003	-34.0 ± 0.8
2 hr	124.0 ± 0.9	147.0 ± 0.3	0.09 ± 0.01	-33.0 ± 0.4
24 hr	121.0 ± 0.5	157.0 ± 0.2	0.06 ± 0.01	-32.0 ± 0.6
Amine				
0 hr	84.0 ± 0.8	82.0 ± 0.2	0.04 ± 0.01	50 ± 0.8
2 hr	151.0 ± 1.4	273.0 ± 0.8	0.03 ± 0.01	-18 ± 0.3
24 hr	187.0 ± 23.0	2023.0 ± 51.0	0.6 ± 29.2	-20 ± 0.4
Carboxylate				
0 hr	91.1 ± 0.6	95.0 ± 0.2	0.02 ± 0.00	-34.0 ± 0.5
2 hr	114.0 ± 0.0	122.0 ± 0.2	0.01 ± 0.00	-24.0 ± 0.4
24 hr	124.0 ± 0.1	122.0 ± 0.3	0.02 ± 0.00	-25.0 ± 0.4

An increase in mean (unmodified, amine, and carboxylate-modified) nanoparticle size is observed following incubation with (10% FBS) for 2 hours

A further increase in particle size was observed at later timepoints (i.e., following 24 hours).

A significant increase in mean particle size was observed for unmodified, amine and carboxylate nanoparticles following incubation for (2, 24 hr).

2) The impact of shear flow on polystyrene latex nanoparticle parameters

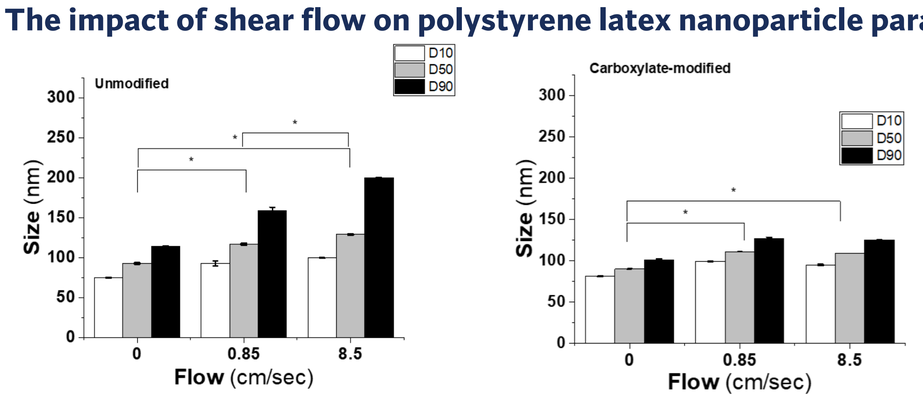


Figure 4. Shear flow impacts nanoparticle parameters following incubation with protein containing media. Polystyrene latex nanoparticles treated with PBS containing 10% v/v FBS under (0.85 cm/s, 8.5 cm/s) shear flow conditions for (2 hours) and isolated using three cycles of centrifugation wash isolation. P<0.05 deemed as statistically significant for a paired t-test. Independent replicated (n=3) with (n=5) measurements per replicate.

- Unmodified and carboxylate-modified polystyrene latex nanoparticles were incubated for (2 hours) at 0.85 and 8.5 cm/s flow rates, mimicking biological shear flow rates in the median cubital vein and arteries, respectively.
- NTA analysis following centrifugation-resuspension isolation shows a significant increase in mean particle size when incubated under increasing shear flow conditions.

3) A comparison of protein composition with SDS-PAGE

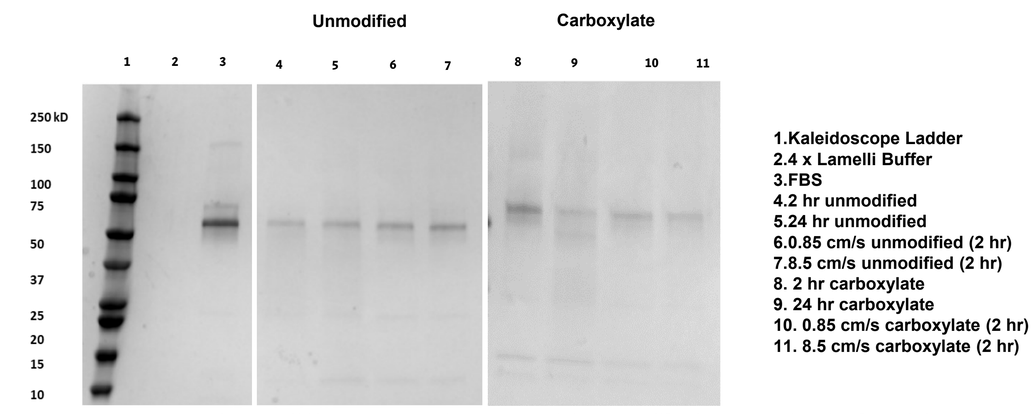


Figure 5. SDS-PAGE shows different protein composition profile for proteins isolated from unmodified and carboxylate-modified polystyrene latex nanoparticles treated with media containing protein under shear versus static conditions. The total amount of sample loaded was normalized to the sample protein content (20 µg per lane).

- The protein composition profile differed between polystyrene nanoparticles with different surface chemistries (~15 kDa band present with carboxylate-modified nanoparticles, but absent with unmodified nanoparticles).
- A band corresponding to albumin observed in all samples (~66 kDa). Additional bands observed at 25 kDa and 12 kDa in unmodified and carboxylate-modified polystyrene nanoparticles.
- The identity and relative quantity of proteins differed in composition as a function of shear flow conditions to which the particles were subjected versus static conditions.

4) The Impact of isolation and shear flow on nanoparticle parameters

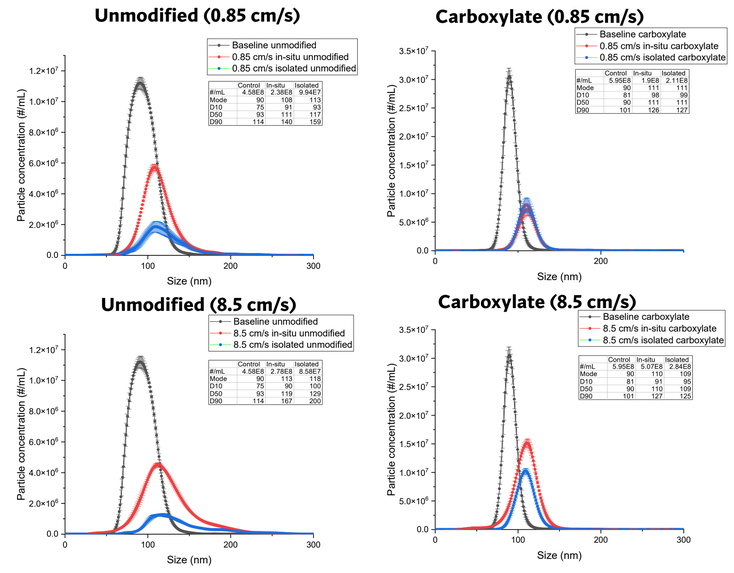


Figure 6. Sample preparation techniques influence nanoparticle parameters following treatment with PBS containing 10% v/v FBS. Unmodified and carboxylate-modified polystyrene latex nanoparticles incubated at (0.85 and 8.5 cm/s) in protein-containing media for 2 hours, measured at baseline and following incubation using (NTA). Baseline traces are represented by (--), in situ analysis with NTA following 2 hour incubation by (--), and centrifugation-resuspension isolated particles by (--), n=3 independent replicates.

- Unmodified and Carboxylate-modified polystyrene latex nanoparticles were incubated for (2 hours) under shear flow conditions.
- NTA analysis was performed as in line in-situ analysis and on nanoparticles isolated using the centrifugation-resuspension technique. We see an increase in mean nanoparticle size following incubation under physiologically relevant flow conditions (0.85 cm/s-median cubital vein) and a further increase at (8.5 cm/s- arterial).
- **Isolation via the centrifugation-wash protocol is highly invasive leading to an increase in mean nanoparticle size due to the centrifugation-resuspension steps, and sample loss at each step of centrifugation-resuspension.**

5) In situ analysis of changes in nanoparticle parameters with AF4-MALS-UV

AF4-MALS-UV was used to analyse unmodified and carboxylate-modified nanoparticles at baseline (left) and following 2 hour incubation (right).

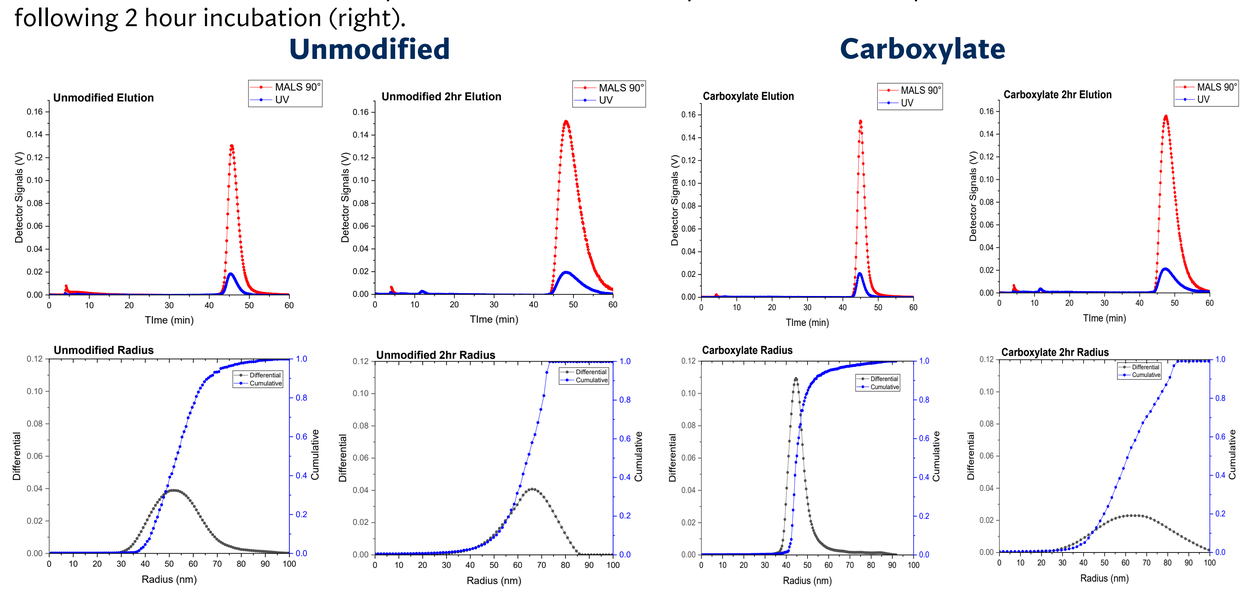


Figure 7. AF4 Fractograms for unmodified and carboxylate nanoparticles at baseline and following (2 hr) incubation. Spacer thickness 350 µm, amphiphilic regenerated cellulose 10 kDa membrane and elution buffer 0.1X PBS.

- A unimodal peak was observed for carboxylate-modified and unmodified particles at baseline and the size data (**figure 7, right**) correlated with NTA and DLS data.
- Following incubation within protein-containing medium, a shift in the elution profile for both particle types was observed along with an increase in particle size from 100 to 131 nm for unmodified, and 100 to 133 nm for carboxylate-modified nanoparticles.
- **AF4 is a gentle separation technique for studying the impact of protein corona formation on nanoparticle parameters.**

Conclusions & Future Work:

- The centrifugation-wash isolation method is highly invasive and leads to an increase in mean particle size and sample loss, limiting the relevance of this approach in studying the protein corona.
- There is an increase in mean particle size when nanoparticles are incubated under shear flow conditions (0.85 and 8.5 cm/s) c.f. static incubations.
- Incubation conditions including duration of incubation and flow conditions lead to changes in the protein corona composition.
- AF4 allows for the gentle separation of nanoparticle-protein samples from protein-containing medium giving us a more accurate representation of nanoparticle size within the biological system.
- Future work will apply AF4 and electric FFF to unmodified, carboxylate and amine-modified nanoparticles.

References:

Jayaram, DT *et al.* (2018) *Biophys J,* 115 (2), pp. 209-216., Xiao, Q *et al.* (2022) *Adv Drug Del Rev*, 186: 114356.

Acknowledgements: We acknowledge funding from Strathclyde EPSRC DTP for this studentship, and EPSRC (EP/V028960/1) multiscale metrology suite for next-generation health nanotechnologies. We thank Dr Yiwen Pei and Dr Caterina Minelli from the NPL for insightful discussions.