Microfluidic methods to investigate particle corona formation

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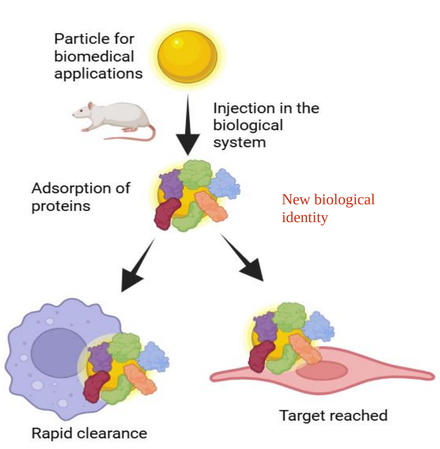
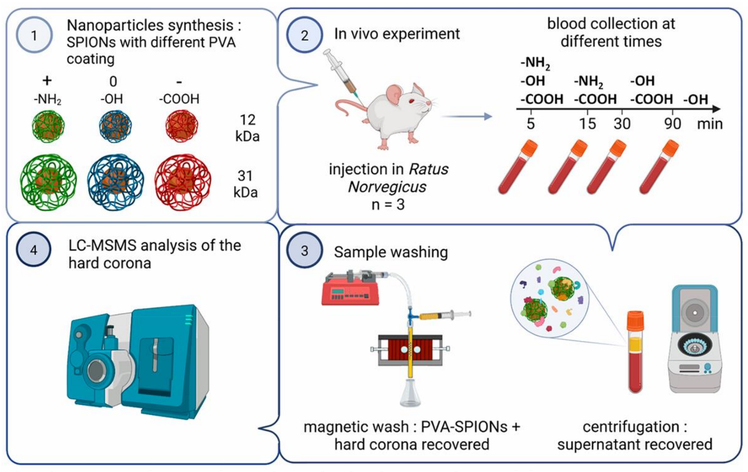
Particles are naturally present in the environment, either formed through natural phenomena (volcanoes, fires), generated by human activities, or even synthesized for various applications in medicine and industry. Because of their presence in the environment, they can easily enter biological system. When particles enter a biological system, they quickly acquire a biomolecular coating called the protein corona [1]. Understanding the formation and composition of the protein corona is essential, as it shapes the biological identity of particles, it determines their safety, effectiveness, and impact in both therapeutic and environmental contexts. We aim to study in real-time this phenomenon using microfluidic technology [2,3].

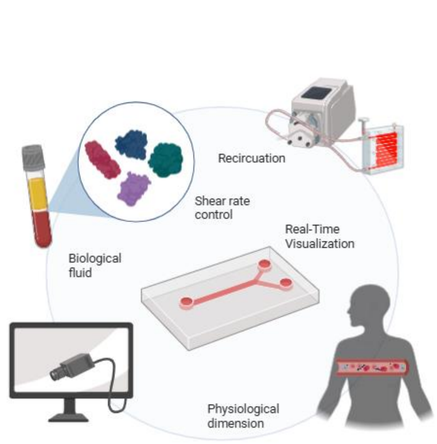




Previous study of corona formation using in-vivo experiments [1]

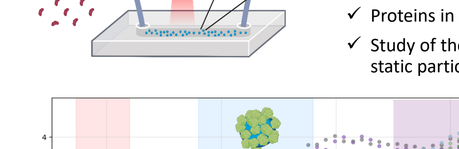
How to characterize in real-time the hard and soft corona formation?

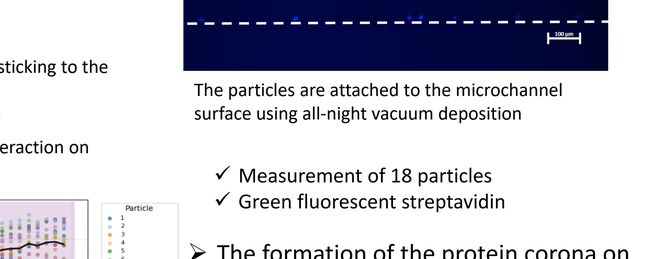
Microfluidics as a tool



Research methodology

Corona formation was measured after incubation using a literature protocol for two proteins: Streptavidin and Albumin.

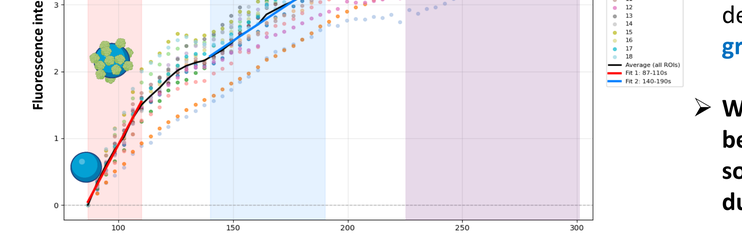




➢ The fluorescence confirmed the formation of a corona for the two proteins on our particles at the end of the incubation

➢ **Experiments far from physiological conditions and not in real time**

Research question: How to observe the corona evolution in physiological condition ?

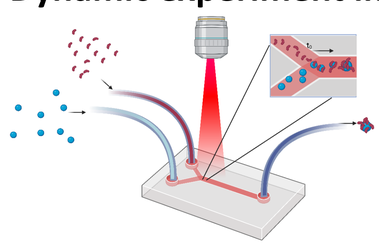


Real time observation of corona formation

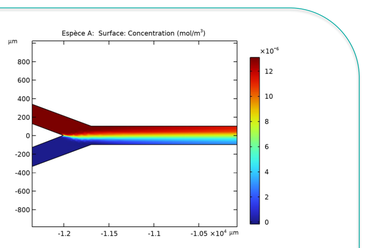
Why microfluidics?
- ✓ Mimic physiological conditions
- ✓ Easy control of flow parameters

Why fluorescence observation?
- ✓ Tracking of individual particles
- ✓ High-sensitivity quantification of local protein concentration [2]
- ✓ Real-time monitoring of particle–protein interaction

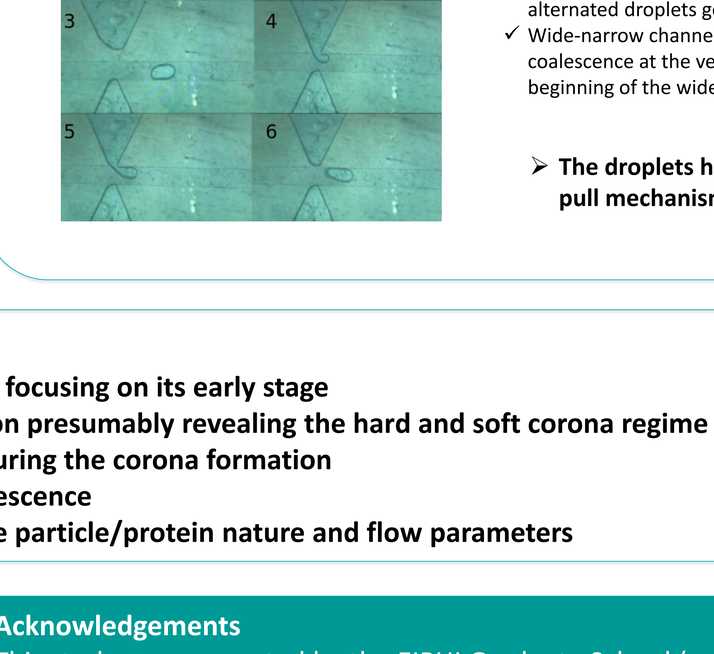
2 Dynamic experiment in channels



- ✓ Particles and proteins in flow
- ✓ Two inlets for co-flowing proteins and particles
- ✓ Simulation for the design of microfluidic circuit
- ✓ Study of the particle and protein join-transport on corona formation



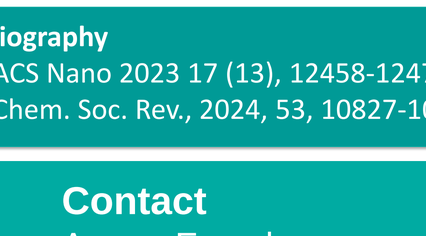
- ✓ Y-branch channel 3cm long
- ✓ Channel size: 50 µm x 200 µm
- ➢ **The laminar flow ensures passive mixing at 5mm/s**



- ✓ The particles are tracked inside the channel using the microscope video acquisition:
- ✓ The increase of the intensity of the fluorescence shows the corona formation around the particle

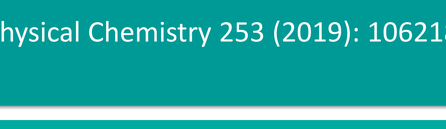
➢ **A distinct dynamic profile emerges under these conditions, in contrast to the static experiment**

1 Static experiment



- ✓ Static particles sticking to the channel walls
- ✓ Proteins in flow
- ✓ Study of the interaction on static particles

- ✓ **Particle deposition**



The particles are attached to the microchannel surface using all-night vacuum deposition

- ✓ Measurement of 18 particles
- ✓ Green fluorescent streptavidin

➢ The formation of the protein corona on different particles shows a three-phase development: **Fast growth**, **Slower growth**, **Stabilization**.

➢ **We speculate the two first phases may be due to the formation of the hard and soft corona [1], and the stabilization due the steady-state regime**

3 Dynamic experiment in droplets

- ✓ Particles and proteins injected inside alternated droplets
- ✓ Droplet coalescence will allow to observe the early stage of the corona formation
- ✓ The same particle will be easily followed along the channel compared to the other configuration

- ✓ Push and pull mechanism in a dual T-junction results in alternated droplets generation
- ✓ Wide-narrow channel induce coalescence at the very beginning of the wide channel

➢ **The droplets have been generated using the push-pull mechanism**

Conclusions and perspectives

- ✓ **The presented microfluidic methods enable real-time observation of protein corona formation focusing on its early stage**
- ✓ **The results of the static experiments show the existence of different phases in corona formation presumably revealing the hard and soft corona regime**
- ✓ **In the dynamic experiment in channel, the preliminary results showed a different behaviour during the corona formation**
- ✓ **The droplets will be loaded with particles and proteins to observe corona formation after coalescence**
- ✓ **Further improvements will target quantitative analysis of adsorption kinetics depending on the particle/protein nature and flow parameters**

Bibliography
[1] ACS Nano 2023 17 (13), 12458-12470
[2] Biophysical Chemistry 253 (2019): 106218.
[3] Chem. Soc. Rev., 2024, 53, 10827-10851

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