High–Throughput–Screening-Fluorescence–Correlation–Spectroscopy for Protein Corona Binding Studies

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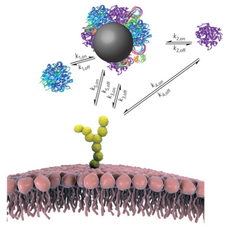
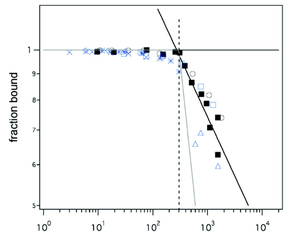
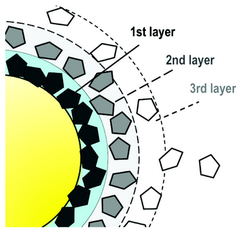
Introduction

The size, structure, and chemical properties of nanoparticles open up a vast range of technical applications and novel approaches. To ensure ourselves of their safety in general, and to apply them as medical tools, it will be necessary to understand how NPs interact with living organisms from a more fundamental point of view. In biological fluids like blood plasma, proteins bind to the surface of NPs to form a coating known as the protein corona, which can critically affect the interaction of the NPs with living systems [1].



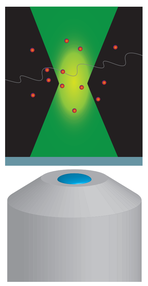
To unterstand the fate of a broad range of different types of NPs when they get in contact with proteins of bioloigical fluids, a high throughput screening approach is needed that uses low sample volumes at realistic conditions and allows fast results.
As Milani et al. showed for transferrin binding to latex NPs, FCS is a suitable technique to study protein–nanoparticle interaction [2].

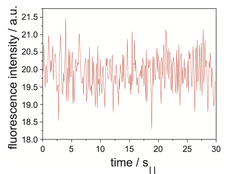




Methods

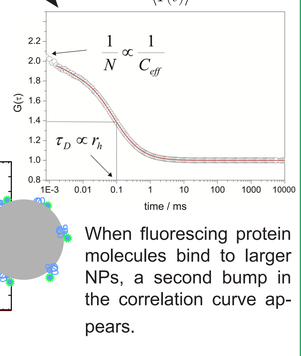
Fluorescence correlation spectroscopy is a powerful single-molecule detection technique that measures and correlates fluctuations in fluorescence intensity within a tiny, confocal volume [3]. The autocorrelation function represents the self similarity of the intensity at time t = 0, and the intensity at all later times. The amplitude of the correlation curve yields the number of particles inside the confocal volume and thus the concentration. The rate of decay of the correlation over time, the so-called diffusion time, τ_D, yields the hydrodynamic radius of the measured particles.

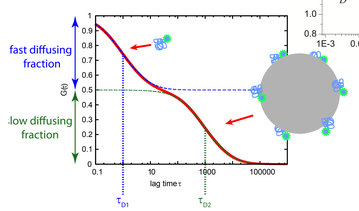




$$G(\tau) = \frac{\langle I(t) \cdot I(t+\tau) \rangle}{\langle I(t) \rangle^2}$$

$$G(\tau) = \frac{1}{\langle N \rangle} \frac{1}{1+\frac{\tau}{\tau_D}} \frac{1}{\sqrt{1+\frac{\tau}{S^2 \tau_D}}}$$





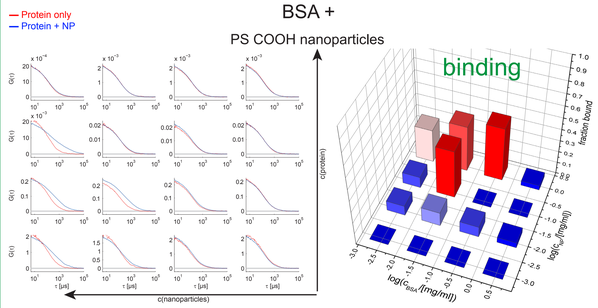
When fluorescing protein molecules bind to larger NPs, a second bump in the correlation curve appears.

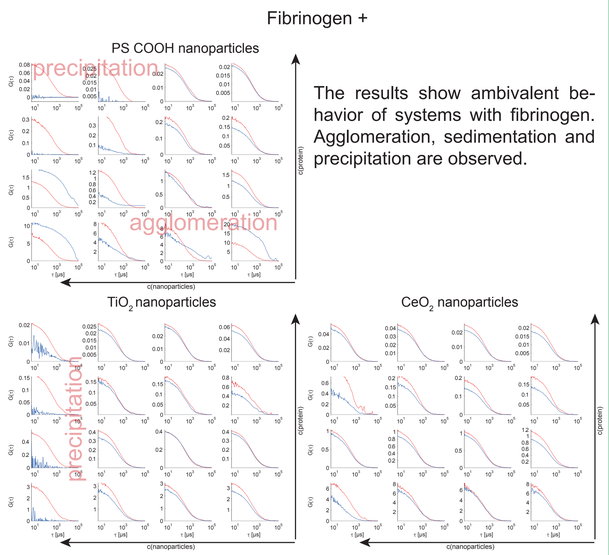
Abstract Protein adsorption to nanoparticle (NP) surfaces is not well understood, although knowledge of the protein corona on NPs in biological fluids is important for nanotoxicity and nanodrug studies and applications. When NPs interact with biological media such as human plasma or serum, proteins and other biomolecules adsorb on the surface. This spontaneous coating gives a biological identity to the NPs, determining their fate within the living systems. In this work, we aim to map the binding of proteins to NPs as a function of both NP concentration and protein concentration using a fast and precise way of high throughput screening Fluorescence Correlation Spectroscopy (FCS) measurements. This allows low sample volumes by combining a liquid handling robot with an automated FCS setup to correlate dose with response in protein-NP interaction studies. Based on the data we can determine the „state of the system“ e.g. aggregated versus dispersed, or the fraction of bound proteins.

Results

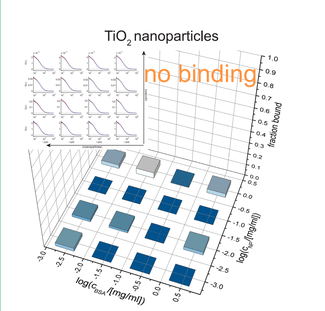
For each data bar two FCS measurements were obtained: the number of initially available proteins is determined first without NP; then the number of remaining free proteins after NP addition and system equilibration is measured. Obtained autocorrelation functions were fitted using a two component function [4]. The fraction of bound protein is analyzed by

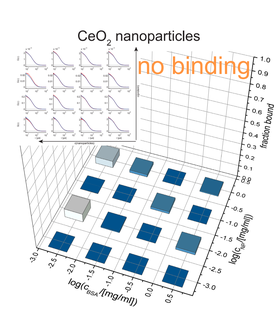
$$\text{fraction bound} = 1 - \frac{N_{\text{free}}}{N_{\text{initial}}} .$$





The results show ambivalent behavior of systems with fibrinogen. Agglomeration, sedimentation and precipitation are observed.

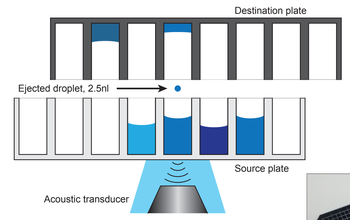


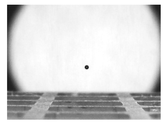


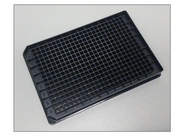
	PSCOOH	TiO2	CeO2	Fe dextrane
BSA	Binding	No Binding, Sedimentation	No Binding, Sedimentation	
Fibrinogen	Precipitation, Agglomeration	Precipitation	to be discussed	
APO	coming soon			
HSA				

Technical Realization

For high throuput screening we automized sample preparation using a contact free droplet dispenser (Echo Labcyte) and a 384 well plate (Greiner Bio One).





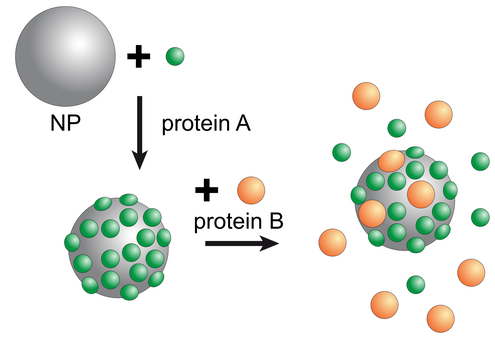


200µl conventional FCS → reduction → 20µl HTS FCS by usage of

Conclusion

We established a high thoughput screening FCS approach that allows studying binding behavior of protein to NPs fast, systematically and in parallel. Preliminary data show different behavior of different types of NPs. States of the system could be distinguished into binding, no binding, sedimentation, agglomeration and precipitation.

Outlook



- Testing further protein – NP combinations
- Examine system behavior in other biological environments e.g. FBS and zebra fish embryo medium
- Competition of two proteins on NP surface evaluating the hierarchy of proteins affinity to the NP
- Time resolved binding essay

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References:
[1] Monopoli, Aberg, Salvati, Dawson (2012)
[2] Milani, Bombelli, Pitek, Dawson, Rädler (2012)
[3] Schwille & Haustein (2009)
[4] Rusu, Lumma, Rädler (2010)

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