ESF – Short Visit Grant - REPORT

Visit 24-28 August of Joachim Rädler at UCD

Project: Transferrin binding to polystyrene Nanoparticles.

Purpose of the visits

The short visit was intended to discuss the progress of experiments carried out in my group in collaboration with Kenneth Dawson (UCD) and Franscesca Baldelli-Bombelli (UEA)

We measured the isotherms of Transferrin binding to polystyrene nanoparticles. Association and dissociation kinetics of TF to/from PS-SO3 in PBS and plasma can be measured using Fluorescence Correlation Spectroscopy (FCS). So far we studied the binding of transferrin in two buffers, PBS and MES.
We achieved the binding isotherms of PS-plain nanoparticles in both buffers, whereas it is possible to observe coating of Transferrin upon carboxylate nanoparticles only in MES. The short visit was used for discussion to establish the framework, experimental conditions and data analysis of FCS measurements. Experiments on sample preparation, biochemical modification and particle characterization will be carried out in Dublin, while FCS measurements are carried out in my lab in Munich.

Achievements

A major issue in protein-NP binding studies is the question of reversibility. The discussions centered around the discrepancy between experimental protocols, where NP are added to protein solution versus adding protein to NP solutions. These various titration pathways in the NP-protein concentration phase space resulted in different apparent binding constants. Protein adsorption to NPs is frequently studied in terms of classical adsorption to solid surfaces. Yet the fact that surface area increases with NP concentration and secondly the fact that adsorption might be irreversible at least for parts of the protein has not been carefully considered yet. Here we show that adsorption isotherms at various protein and NP concentration all fall onto on universal curve, if plotted as a function of protein-to-NP ratio. Using fluorescently labelled transferrin as a model system we measure the fraction of bound protein using fluorescence correlation spectroscopy. The adsorption curve shows existence of a strongly bound monolayer (hard corona) and a weakly bound secondary layer (soft corona). While the first layer is shown to be irreversibly bound the secondary layer follows a Langmuir isotherm.

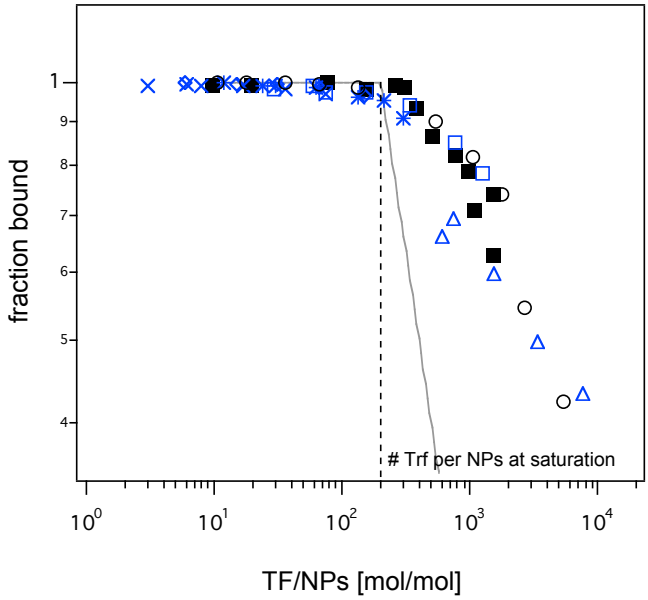

Figure A normalized representation (fraction bound vs the ratio of protein to NP) shows a universal behaviour. The vertical dashed line indicates the ratio of full surface coverage. The gray line represents the theory of a single stringly bound monolayer as discussed in the text Titration of NPs to Trf (black symbols) or Titration of Trf to NPs (blue symbols).

Manuscript in preparation

Measuring the number of proteins binding to nanoparticles by FCS

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