SCIENTIFIC REPORT: PROTEIN CORONA EVOLUTION AND ITS EFFECT ON NP UPTAKE INTO CELLS

This report includes the result obtained during my short visit to The Centre for BioNano Interactions (CBNI) at University College Dublin (UCD) from March 9th to March 12th. My purpose for this visit was to perform a control experiment for a project that is in its final stages and was started during my time as a post doc at CBNI. The experiment aimed to investigate if previously internalized nanoparticles (NPs) would affect subsequent uptake of additional NPs.

As this was a single type of experiment carried out over four days I will here describe what I was doing during those four days:

Friday 9th: Cells (A549) were seeded in 3x6-well cell culture dishes, 150.000 cells/well. Plates were stored in incubator (37 degrees, 5% CO2) over night.

Saturday 10th: The experiment: NP-dispersions and medium were prepared before the start of the experiment. Four samples time three replica and three control samples were going to be performed. The NPs were 100 nm, carboxylated polystyrene NPs (both fluorescent and non-fluorescent types were used).

- Sample 1 (UL9): 9 pulses of unlabeled NPs before one final pulse of fluorescently labeled NPs.
- Sample 2 (UL8): 8 pulses of unlabeled NPs before one final pulse of fluorescently labeled NPs.
- Sample 3 (UL1): 1 pulse of unlabeled NPs before one final pulse of fluorescently labeled NPs.
- Sample 4 (UL0): 0 pulses of unlabeled NPs before one final pulse of fluorescently labeled NPs.

Each pulse was 10 minutes long and between each pulse there were a 45 minutes chase time (time in medium without NPs). Each time the medium on the cells were changed (to either contain or not contain NPs) the cells were washed with phosphate buffer. After the final pulse the cells was left in the incubator over night with NP-free medium.

Another set of plates were seeded in the same way as Friday the 9th. These were for the same experiment but with 40 nm, carboxylated, polystyrene NPs.

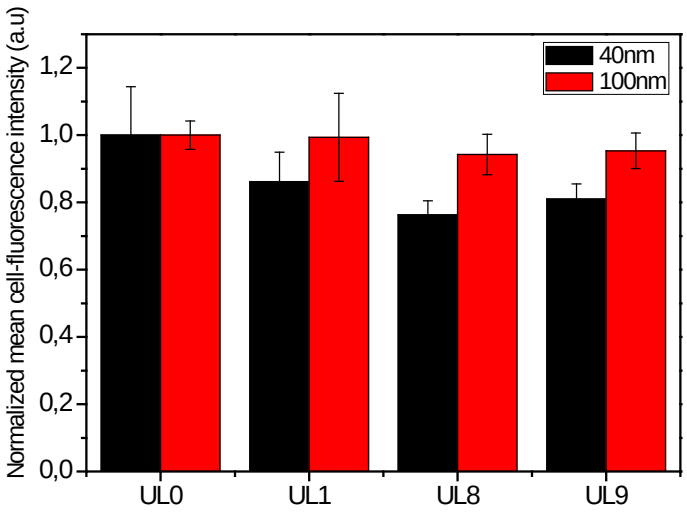
Sunday 11th: The same type of experiment performed the day before was performed again but with the 40 nm NPs.

In addition, all 100 nm samples were prepared for analysis using flow cytometry. Samples were measure using a Dako CyAn-ADP flow cytometer

equipped with a 488 nm laser. A total of at least 15.000 cells were measured for each sample.

Monday 12th: The 40 nm samples were analyzed in the same way as the 100 nm samples the previous day.

The results were analyzed by comparing the median cell fluorescence (which is proportional to the amount of fluorescent NPs taken up by the cell) in each population of cells. Each sample consisted of three replica and the results were tested using the One-way ANOVA statistical analysis with the Bonferroni comparison test.



The graph is showing the normalized average cell-fluorescence intensity which corresponds to average uptake of the fluorescent NPs per cell. UL stands for unlabeled and the number describes the number of pulses of unlabeled NPs preceding the final pulse of fluorescently label NPs, i.e. UL8 means that 8 pulses of unlabeled NPs was added to the cells before one final pulse of fluorescently labeled NPs was added. Each sample is an average of three replicas and the error bar shows the standard deviation. Black and red columns show the result for the 40 nm and 100nm carboxylated polystyrene NPs, respectively. Statistical analysis using One-Way ANOVA with the Bonferroni comparison tests showed that there is no statistical difference between any of the samples at the 0.05 level.

The results showed that there is no statistical difference in NP uptake when comparing cells with no previously internalized NP to cells with a significant amount of previously internalized NPs. This means that intracellular NP does not affect continued uptake, at least up to the amount used here. The result was what we were hoping for and helps to strengthen our conclusions in the rest of the paper.

This work is being written up however, more experiments may be necessary before publication is possible in which case it will be done in collaboration with the host institution (CBNI). Although there are no specific plans at the moment there is a distinct possibility for future collaboration in other projects as I am continuing work on NPs and cell interactions.