SCIENTIFIC REPORT :The Influence of Biofunctionalisation of Nanoparticles on the Nature and Dynamics of the Nanoparticle Protein Corona

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The purpose of this visit was to become integrated within the CBNI group at UCD in order to understand their way of approaching and tackling the issue of bionanomaterial preparation and evaluation as potential nanomedicinal agents. As my relevant background is in nanoparticle synthesis and functionalisation the aim was a mutually beneficial stay with information and experience transferred both ways.

As a large multidicsciplinary group spanning biology, computation and chemical synthesis the work was performed with the aim of utilising the various expertise present.

Two fronts were tackled

- Fluorescent Particle detection by Organic Dye or Quantum Dot Encapsulation. Looking at introducing QD materials as fluorescent markers [1].
- Optimisation of Transferrin and Albumin nanoparticle functionalisation for cellular uptake and in vivo studies.

The first process involved optimisation of microemulsion silica nanoparticle syntheses incorporating synthesised[2] dodecylamine capped 6nm goldNPs(more economical for synthesis optimisation) or ZnS/CdSe QDs. A second consideration was the dispersibility of the composites, with a consequent surface bioconjugation step being the goal monodispersity was crucial.

Preparations were run with the particles washed by centrifugation and then characterised by DLS, UV, TEM and Fluorimetry.

The biofunctionalisation work was carried out on commercially available 100nm polystyrene particles in order to optimise procedures and practices with quality and reproducibility was of vital importance considering biological studies.

Various preparations and purification techniques were tested with satisfactory particles in terms of monodisperity in relevant media and protein amount covalently

bound then forwarded for biologcal testing on dot blot arrays and cellular uptake studies.

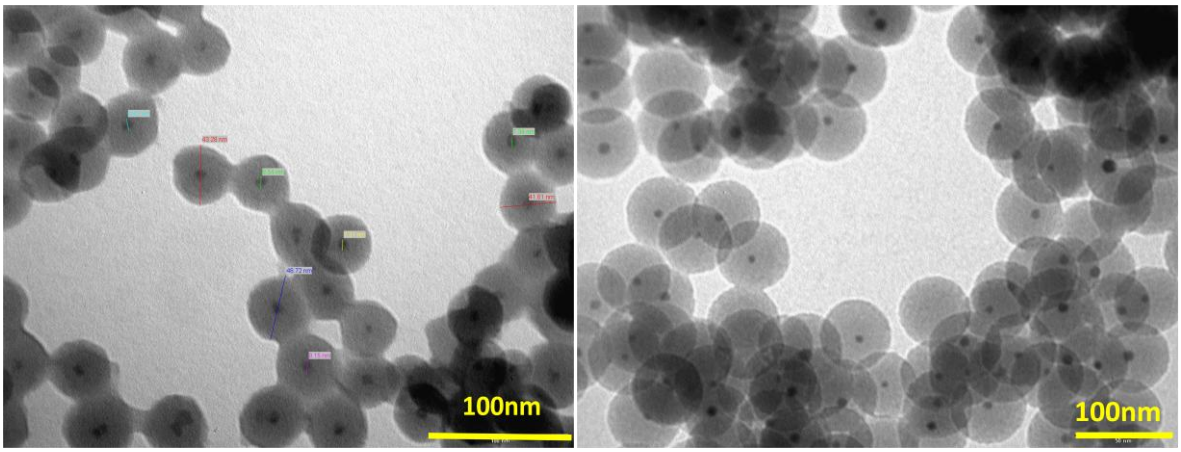

Fig. 1 TEM of Si-QD composites and Si-AuNP composites

On the nanoparticle synthesis side 40 and 60nm silica nanoparticles containing one quantum dot per particle were successfully prepared similar to reported procedures[3] and rendered monodisperse through surface functionalisation with charged groups similar to reported procedures.[4] Also successfully prepared were 40nm silica NPs encapsulating 5nm gold.

Fluorescent Dye Silica nanoparticles were also prepared with preparation of Dye-Silane conjugates which were then incorporated in the SiNP framework by co-condensation with the silica precursor TEOS in a microemulsion. These particles were mondiperse and showed diameters fro 20-40nm in TEM.

Both of these particles display amine groups amenable to further conjugation with biomolecules.

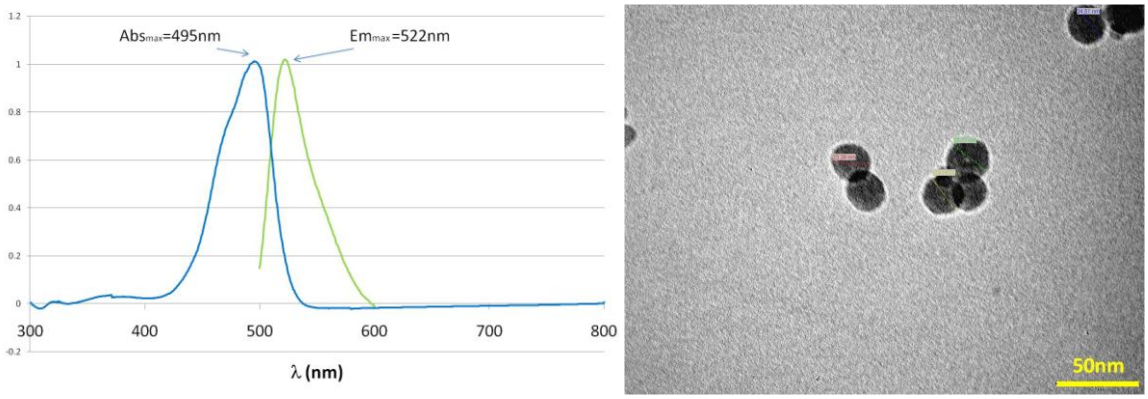

Fig. 2 Si-FITC NPs Absorption and emission spectra (left) and TEM image (right).

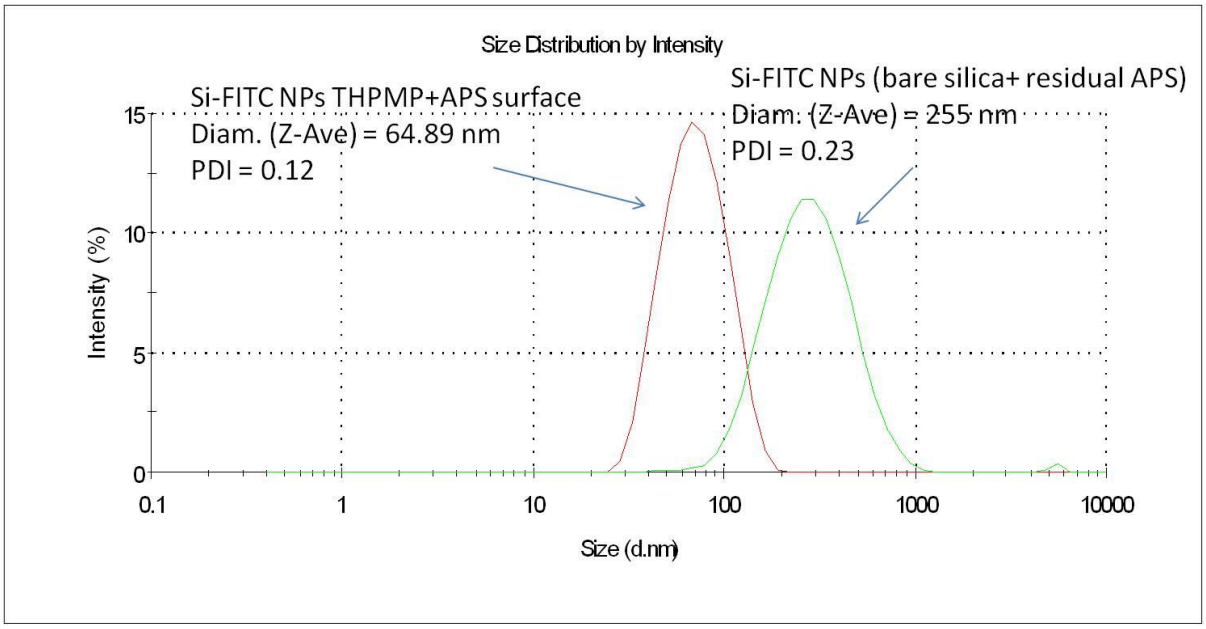

Fig. 3 DLS measurements showing effect of surface functionalisation (phosphonate + amine) on particle dispersity in water.

Optimal Procedures for Polystyrene NP-Protein conjugates were uncovered maintaining monodispersity and displaying a protein surface. Carbodimide chemistry was used to couple the caboxylate presenting particles with free amino groups on the protein surface.[5] A one step EDAC based reaction[6] as well as a two step EDAC-NHS coupling were used .[7]

The prepared particles were examined by DLS, protein assay and dot blot assays to investigate size, monodispersity, protein numbers and biorecognition activity. The effect of Transferrin decoration on particle behaviour [8] is part of ongoing work using these particles with a view to optimising efficacy through directed protein orientation.

Characterisation of protein adsorbed on particles is challenging as the traditional protein spectroscopic techniques meet limitations due to particle scattering effects. The biological activity of such particles can be investigated using dot assay techniques or surface biosensor measurements. Preliminary QCM measurements[9] have been instigated at Montpellier using bilayer immobilised receptor to look at the nanoparticle biological affinity in a biomimetic way.

As of the now no publications/articles have been submitted but are in process with interesting results pertaining to transferring functionalised nanopaticle uptake

pathways. The final steps are to bring together the platform particle synthesis and the bioconjugation and purification.

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