Differential Centrifugal Sedimentation for the Advanced Characterisation of Nanoparticles: Probing the Bio-Nano Interface

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Outline

- Gold nanoparticles for biomedical applications
- Functionalisation of nanoparticles
- Characterisation of functional nanoparticles
- Role of DCS – high resolution characterisation
- Probing the bio-nano interface §
- Biomolecular coronas & Biological identity of NPs
- Mapping protein binding sites on biomolecular corona of nanoparticles
- DCS – further challenges
Roadmap Towards the Future of Nanotech

Key enabling element for wider use of nanotechnology in medicine: Nanoparticle Characterisation
Quality control - challenges

- Batch to batch reproducibility
- Scaling up
- Characterisation of pristine surfaces
- CLEAN synthesis

- Characterisation in relevant conditions
- Biological testing
- Environmental

Particle Synthesis

Particle Dispersion

Validation

Biological Testing

- Experimental protocols
- Positive and negative controls

CLEAN synthesis
Why Gold Nanoparticles?

- Extreme stability
- Versatile preparations – monodispersity and NP libraries
- Ease of functionalisation
- Relatively non toxic
- Highly visible by EM and optical methods
- Raman enhancement
- Local laser heating (hyperthermia)
- ...

GNPs

200 nm
Functionalisation

Top reasons to use GNPs for biological applications:

- DNA, gene delivery
- Drug delivery
- Intracellular surgery
- Imaging/Tracking in cancer therapy
- Therapy
- Protein – receptor study
- Detection / Sensing

Characterisation of functional NPs for biomedical applications

Preparative Nanochemistry

Materials characterisation (core particles)
- UV-vis (plasmons/concentration)
- DLS (rough estimate)
- Z-potential
- DCS (monodispersity, d)
- Nanosight (number density)
- Weight concentration (ultra balance)
- Surface
- TEM (morphology, monodispersity)
- \textit{BET (or alternative) – surface area}

Functionalised Materials characterisation
- UV-vis
- Z-potential
- \textbf{DCS} (shift due to coating, dispersion stability)
- NMR
- \textit{ATR FTIR}
- \textit{Specific Functional Group assays}
- \textit{Gels (EP, SDS-PAGE)}

Quantitative NP Concentration:
- UV-vis for Molar conc
- Ultra balance for weight concentration
- Nanosight for number density

Advanced Characterisation

Characterisation prior to Biological applications
- Supernatant chemical analysis (endotoxin assays)
- Centrifuge the particles and analyse for trace chemicals (MS)
- Dispersion in biological media used in particular application (DCS/ HS, HP or Cell culture medium)

Nanodiagnostics & Sensing

Therapy & Drug Delivery

Uptake Studies
Stokes equation – Theory

V = D² (ρ_P – ρ_F) G / 18 η

D the particle diameter (cm)
ρ_P particle density (g/mL)
ρ_F the fluid density (g/mL)
G the gravitational acceleration (cm/sec²)
η the fluid viscosity (poise)

Advantages over other Particle sizing techniques:

- High resolution
- Accuracy & Precision
- Sensitivity
- Dynamic range
- Analysis of the entire sample with rapid detector response
How does it work?

Small molecules: citrate, BSSP, functional PEG thiols, DNA
Peptides: CALNN, CALNNAAA[AAAAE]₃AA
Proteins
Other NPs

Increase of ‘real’ NP diameter: functionalisation

Decrease of GNP density 19.3 g cm⁻¹ \(\geq\) <<19.3 g cm⁻¹

Decrease of ‘apparent’ NP diameter

Gold hydrosols are increasingly used for applications that require the precise control of particle core-size and ligand shell thickness.

Our model showing how small changes in ligand shell impacts the overall particle size on DCS...
DCS – Detection of dimers/trimers *in situ*

Formation of DNA-Polyamide interaction induced aggregates

dsDNA detection using Py-lm Polyamide functionalised GNPs

Target sequence: 5’WWGWWWCW3’
W=A/T

*e.g.* 5’ TAGTACT 3’


DCS – Detection of dimers/trimers *in situ*

Target sequence: 5’WWGWWCW3’
W = A/T
Specific detection of dsDNA using Pyrrole-Imidazole functionalised GNPs

PA sequence: Im-Py-Py-Py-Im-Py-Py-Py

Au-ODN sequence: SS-(CH2)$_6$-(HEG)$_3$-5'-GCT GCT TAG TAC T-3'

Fully matching ODN: 3'-CGA CGA ATC ATG A-5'

The Biological Context of Nanoparticles

- In biological fluids the identity of the nanoparticle is altered.
- Adsorbed molecules determine confer a biological identity.
- The challenge is now to understand and control the biological identity.
Biomolecular Corona

NP → NP@Protein corona

Dynamic corona

Hard corona

Time

Dynamic process - evolves over time

A. Salvati et. al., *Nat Nanotechnol*. 8, 2013, 137.
Human plasma proteome

Protein abundance not evenly spread
Stability in Cell Culture Medium - DCS

Biomolecular corona formation in biological media

- UV vis.
- Stability in Cell Culture Medium - DCS
- Biomolecular corona formation in biological media

GNP 1 = 5 nm
GNP 2 = 10 nm
GNP 3 = 15 nm
GNP 4 = 40 nm

(a) Absorbance (Normalized) vs. Wavelength (nm)
(b) Relative Particle Number vs. Diameter (μm)

Water
1 hr DMEM
4 hr DMEM
Transferrin Binding Kinetics

ρ (Au) = 19.3 g/cm³
ρ (PS) = 1.07 g/cm³

Sucrose density gradient:
8-24%
2-8%

Transferrin coated Au and PS NPs

8.9nm  11.8nm
227nm  275nm
Mapping Protein Binding Sites on the Biomolecular Corona of Nanoparticles

Kelly, P. et al., Nature Nanotechnology, 2015, 10, 472-479
Assessing bio-nano interactions

- If you’re a chemist you can work out a Chemical Structure
- If your interested in biologicals, you can do X-Ray Crystallography
- For Drugs you can co-crystallise and look for drug binding sites.
- For nanoparticles this is not possible as coronas lack structural coherence necessary to gain crystallographic data.
- We need alternative approaches

Das et. al. Nature Structural & Molecular Biology 19, 2012
Visualising the Biomolecules

Model Particles
- Polystyrene (low density)
- 200nm (Easy to image)
- Low electron density
- Good stability and size distribution.

Immunogold
- Au core 5nm
- Antibody, adsorbed.
- Remaining surface blocked with BSA

Different Abs – different address labels
Immunogold Labelling Approach

- Using immunogold we can label epitopes on the surface of nanoparticles.
- Identify Target
- Progressive binding can be monitored using DCS

Epitopes:
- TfR- Yellow. (aa. 122-125)
- Monoclonal Green. (aa. 142-145)
Visualising the Immunogold

Visualise the epitopes of biomolecules using TEM

Polystyrene is semi-transparent to the electron beam.

We see all the gold whether its top or bottom.
Particle diameter shift

30 min saturation is reached
1h – Tf corona formation – hard corona – stable

Transferrin Binding Kinetics

Particle diameter shift – monitoring saturation of the protein sites

Increasing IG@ATf concentration
Protein Binding Sites Mapping on the Biomolecular Corona of NPs

200 nm sulphonated PS

4 nm in-house made GNPs ATf antibody conjugated

*Slot-Geuze method*

Using a Calibration Standard

\[ D = \frac{D_c^3 \rho_c + (D_s^3 - D_c^3)\rho_s - D_s^3\rho_f}{D_s(\rho_c - \rho_f)} \]

Following Immunogold Labelling

Construction of binding curves, addition of IG makes the particles heavier and appear larger in size – diameter is false but useful to follow the binding.

\[
D_{app}^2 = \frac{D_c^3 \rho_c + (D_s^3 - D_c^3) \rho_s - D_s^3 \rho_f}{D_s(\rho_c - \rho_f)}
\]
How many do we have?

- We can count each condition, however the most interesting events are at saturation.
- Roughly 260 are labelled by mTf.
- ~10% of the Transferrin.
- 40 on an 80 nm particle.
- Approx. 1 per 13nm particle.

Relative Shift (nm)

Immunogold label concentration (nM)
How they are distributed?

Started with the distribution of pairwise distances.

This distribution can be solved for 2D projected images.

For this system the distribution is random.
Visualising in 3D

- Using STEM we can reconstruct the 3D placement of the labels.
Profiling the Biomolecular Corona

Looking at real corona system: Tf & IgG
Population analysis = relative composition by Mass Spec
Single particle imaging – to look at protein distribution

- DCS allows us to look the population.
- Single particle analysis shows the individual biological Identity
Challenges for DCS

- Anisotropic nanoparticles
- Ultra-small functionalsed nanoparticles
- Using the DCS as a fine detection tool
DCS for LPS detection – Towards endotoxin free NP

Dose dependent shift
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http://www.salford.ac.uk/research/brc