



Neutron research in nanoparticles for medicine

Nanotechnology is usually defined as science, engineering, and technology conducted at the nanoscale, which is about 1 to 100 nanometers ([National Nanotechnology Initiative](#)). There are many different types of nanoparticles, made of different materials, and in a wide variety of sizes and shapes. Typically, their size ranges from a few nanometers (nm) to hundreds of nanometers. On the atomic scale, atoms are separated by 0.2 - 0.3 nm, so one nanometer corresponds to 3 to 5 atoms in a row, or somewhat less than 100 atoms in a cube with 1 nm side. A 100 nm sphere already has millions of atoms.

Nanoparticles, with size somewhere between the atomic scale and bulk material, often have interesting and sometimes unexpected properties. A large proportion of the atoms is at the surface, which behaves differently than the bulk. Size matters, and for instance suspensions of gold nanoparticles can be red, black, or violet, but not yellow or golden, depending on the nanoparticle size.

Organic coatings, including DNA, are easily attached to gold nanoparticles (AuNP), leading to hybrid organic/inorganic nanomaterials. In medicine, most interest in functionalized AuNPs is in their use as drug carriers: the small size of the nanoparticles means they can penetrate cells, where they can deliver the organic material. Other uses include the detection of biomarkers for screening of diseases such as cancer, and also bio-imaging.

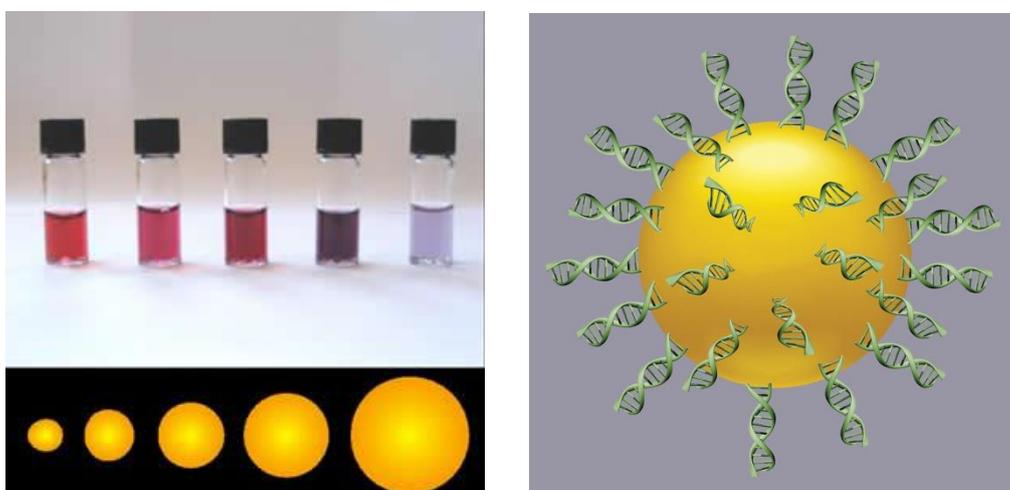


Figure 1. Left: Suspensions of gold nanoparticles of different sizes (image credit: Wikipedia). Right: A functionalized nanoparticle.

In biomedical applications the size and shape of the nanoparticles is important and needs to be controlled, with low tolerance for batch or vendor differences. Christopher B. Murray (University of Pennsylvania, USA) and collaborators studied a series of hybrid AuNPs, where the organic layer thickness was gradually controlled from 1.2 nm with the common commercial ligand dodecanethiol (L = DDT) to 4.1 nm by the synthesis of disulfide dendritic molecules with one (L = G1), two (L = G2), and four (L = G4) generations ([Benjamin T. Diroll et al., 2015](#)).



Conventional electron microscopy and X-ray techniques can only provide information on the inorganic cores of the nanoparticles, but not on their organic coating. Using Small Angle X-ray Scattering (SAXS) Murray and co-workers could determine the size of the gold core of the nanoparticles. Small Angle Neutron Scattering (SANS) is a similar technique that uses neutrons instead of X-rays, and with it they measured the size of the organic coating (cf. Figure 2). An important finding was that higher dendritic generations lead to increasingly compact molecules, denser and less compressible. The combination of SAXS and SANS measurements provided nanometer-scale size resolution for hybrid nanomaterials, and also offered a way to study their response to external stimuli.

Wenlong Cheng (Monash University, Australia) and collaborators showed, using SANS, that DNA-capped AuNPs responded differently to changing ionic strength and temperature ([Wenjuan Yang et al., 2015](#)). Palindromic DNA (a type of DNA often found in genomes or sets of genetic instructions) hybridize at high ionic strength, resulting in aggregates of large numbers of connected DNA-capped nanoparticles. Changes in temperature could control the process. Their results showed that it is possible to guide the design of tailor-made DNA corona structures for customizable designer materials. For instance, DNA density directly affects the cellular uptake efficiency of nanoparticles, which is one of the most important factors in targeted delivery of drugs.

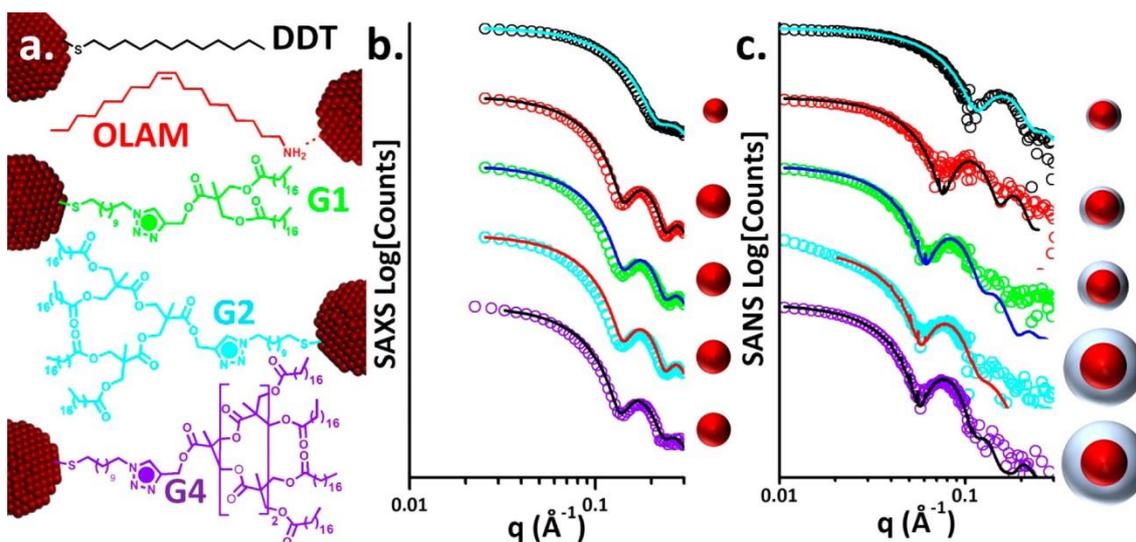


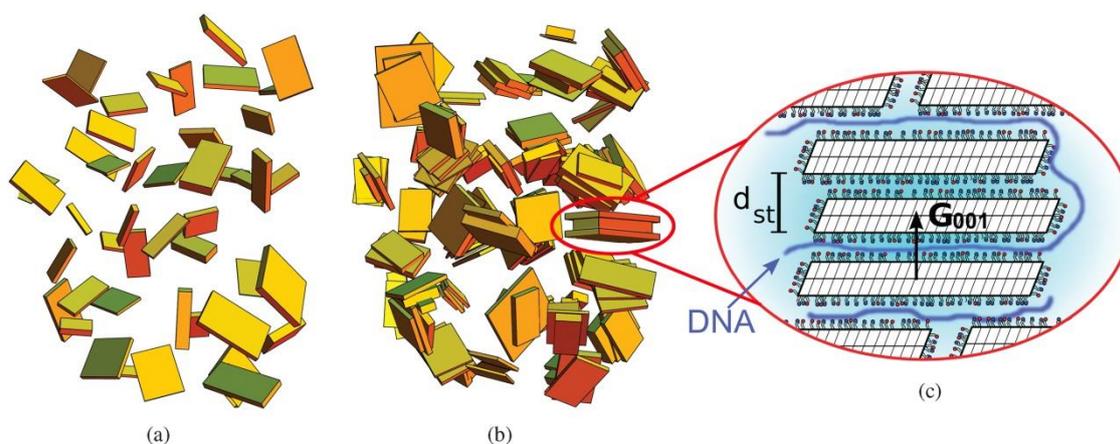
Figure 2. a) Ligands with thickness from 1.2 nm dodecanethiol (DDT) to 4.1 nm by the synthesis of disulfide dendritic molecules on oleylamine-stabilized nanoparticles (OLAM) with one (G1), two (G2), and four (G4) generations. b) SAXS data. c) SANS data. Figure credit: [Benjamin T. Diroll et al., 2015](#).

A very different type of nanoparticles is being studied for drug delivery systems since the 1990s: solid lipid nanoparticles (LNP), which allow the administration of poorly water soluble drugs and can prolong their circulation time in the body. Specific ligands grafted at the surface of the LNPs promote the docking of the LNPs at the matching receptors of the target cells.

One of the outstanding issues in gene therapy is transfection, i.e. how to efficiently insert DNA into cells. One method is to use viral vectors, in which a piece of DNA is attached to a virus capable to penetrate the cell membrane. This poses potential safety concerns, so non-viral transfections vectors are a desired alternative.



Tobias Unruh (Friedrich-Alexander-Universität Erlangen-Nürnberg, Germany) and co-workers used SANS, SAXS, and other techniques, to study cationic LNPs (cLNP) ([Martin Schmiele et al., 2015](#)). These can be produced on a large scale with physiologically well tolerated ingredients without using organic solvents. The cLNPs were stabilized in suspensions, forming platelet-like nanostructures, ranging from the sub-nano to the micron scale. When DNA is added to the suspension, nanocomposite structures with a sandwich-like arrangement of the DNA and platelets are formed, where the DNA is intercalated between platelets. These nanocomposites could be of interest as DNA carriers, since the sandwiched DNA is protected from degradation. The combination of techniques, in particular SAXS and SANS, was essential to build a structural model of the nanocomposite structures.



Schematic illustration of the cLNP suspension (a) before (only individual cLNPs) and (b) after addition of DNA (DNA induced stack formation). c) Structural model for the DNA intercalated between the cLNP platelets. Figure credit: [M. Schmiele et al., 2015](#).

Oral administration of drugs is the preferred and easiest method, but many drugs have low oral bioavailability. One of the main causes is the mucus barrier in the gastrointestinal tract, which guarantees the absorption of nutrients while preventing the permeation of many pathogens and foreign particles. Drug delivery systems including micro- and nanoparticles are trapped in mucus layers, and in many cases particles do not reach their target at all.

One way to overcome this is to design nanoparticles with increased mucus-permeating properties and increased residence time in deeper mucus layers where the particles get close to the absorption membrane. The strategy is to decorate the nanoparticles with mucolytic agents able to cleave the mucus substructure.

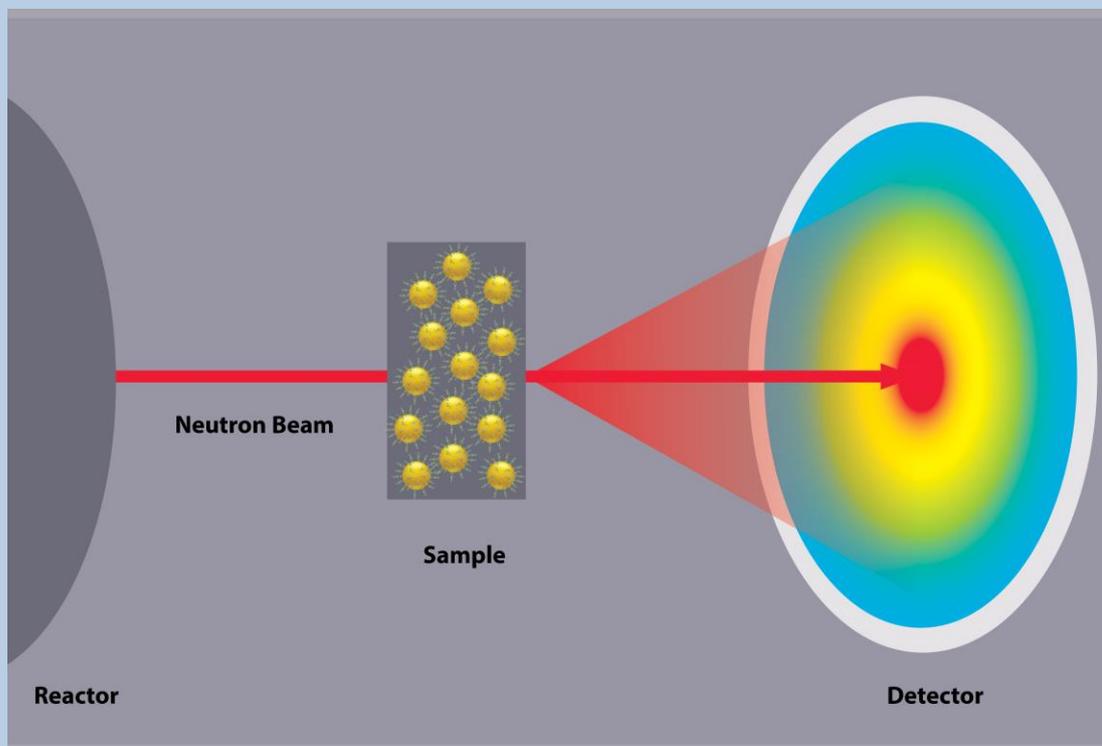
Andreas Bernkop-Schnürch (Leopold-Franzens-University of Innsbruck, Austria) and co-workers used SANS, SAXS and other techniques to study the permeation ability of nanoparticles, either bare or decorated with different enzymes, in intestinal mucus ([I. Pereira de Sousa et al., 2015](#); see [Julia Griebinger et al., 2015](#) for a review including the capabilities of a host of different analytical techniques). The bare particles showed limited mobility, which was associated with their lack of capability to perturb the mucus structure. On the contrary, the surface decorated particles induced a localized destruction in cross-linking density, leading to their greater mobility through the mucus associated with a greater mobility of the mucus itself.

What is Small Angle Neutron Scattering?

Small Angle Neutron Scattering (SANS) is a technique similar to Small Angle X-ray Scattering (SAXS). A collimated neutron beam is deflected as it crosses a sample and interacts with the structures within it. The resulting deflection can be a fraction of a degree, up to 10° .

The interaction depends on the size, shape and orientation of the structures, and so these properties can be studied with SANS and with SAXS. The length scale that is studied ranges normally from 1 to 100 nanometers, which matches the size of many biological systems such as proteins, viruses, biological membranes, and also nanoparticles.

X-rays are more sensitive to heavier elements such as metals, so SAXS can be used to study e.g. the size and shape distribution of gold nanoparticles, which are used in many applications. Neutrons are more sensitive to light materials such as hydrogen or carbon, typically found in organic materials, and SANS is ideally suited to study the organic coatings of functionalized hybrid organic/inorganic nanomaterials.



Transmission electron microscopy (TEM) is one of the most common methods for characterizing inorganic nanoparticles. However, it has reduced contrast for organic material and therefore it is not very adequate to observe the organic layers on the surface of inorganic nanoparticles.