# STUDENT BACKGROUND READING FOR EXPERIMENT C:

# COLORIMETRIC GOLD NANOSENSOR

In this experiment you will synthesize and test a *plasmonic colorimetric nanosensor made of nanoparticles of gold*. Here we provide you with some background information to make the most of this experiment.

## What do you know already about gold?

Think of gold as you know it. Gold (Au, atomic number 79) is the most malleable and ductile metal of all; it can be beaten to very thin sheets of material and rolled or bent as desired. This has been known and done for centuries. The **colour of pure** gold is metallic yellow ("golden"). You have probably seen or heard of "rose gold" or "white gold", but these are not made of pure gold; these are *alloys of gold*, they contain other metals such as copper and silver.

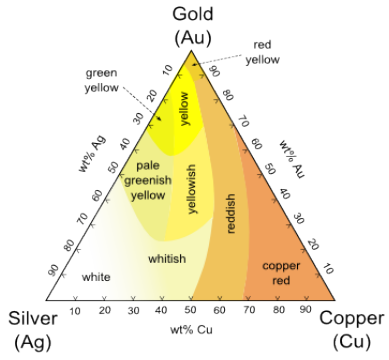


**Figure 1**. Approximate colours of Ag-Au-Cu alloys. (Image credit: Wiki commons, Creative commons Attribution ShareAlike 3.0)

**What about gold´s reactivity?** Gold is **very stable and not-toxic**, and for this reason is widely used in jewellery and dentistry because is air-inert and is not affected by most chemicals. Gold is also a **good conductor of heat and electricity** (which is due to the fact that conduction electrons are free to move around the nucleus); it is **corrosion resistant** so it is used for electronic contacts and other electronic applications. Gold has also numerous other applications: for instance, thin layers of gold (so thin as to become transparent) are applied to the windows of large buildings to increase the amount of sunlight reflected by the window. This way, less air conditioning is required in summer to keep the building cool.

**In this experiment we investigate nanoparticles of gold (or "nano-gold").**



The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement n° 233433

## Is nano-gold different from the gold we are familiar with?

Yes it is! The properties of nano-gold are very different and it all has to do with the size of the nanoparticles.

When gold nanoparticles are inside a medium, such as water, they create a **colloid**. A colloid is different from a solution. A *solution* is a chemical mixture where a substance is evenly dispersed in another one (such as a salt solution); a *colloid* is another type of chemical mixture: the particles of the dispersed substance are only suspended in the mixture, they are not completely dissolved in it. This occurs because the particles in a colloid are larger than in a solution. A colloid is composed of particles in the range of 10-100nm. Gold colloids can have many different colours, ranging from ruby-red, to purple, to blue. And these are not alloys, they are made of pure gold nanoparticles! A characteristic of colloids is that they **scatter light**.

**Colloids exist in nature** and can be in the form of emulsion (such as milk), gel (gelatine), aerosol (fog), and many other forms. Even custard is a colloid! Where are the nanoparticles in these materials? For instance, milk is an emulsion of macromolecules (casein micelles and liposomes) in water. Casein micelles and liposomes are a type of natural nanomaterial.

A simple way to **test if a mixture is a solution or a colloid** is to shine a laser beam though the mixture: the light will be scattered only by the colloid. To be visible, the colloid must be relatively transparent. For instance milk is a natural colloid but it is opaque. So to perform this test you need to dilute the milk. **WARNING**: never shine a laser beam near the eyes nor look straight into the beam!

## What determines the colour in gold colloids?

Metal nanoparticles, including gold nanoparticles, have optical properties which are very different from the properties of the corresponding bulk ("macro") material. This is due to an effect called **localised surface plasmon resonance (LSPR).** In a conventional metal, electrons are free to move in all directions. When a beam of light (an electromagnetic radiation) hits the material, the energy is absorbed by the conduction electrons, which start to oscillate, generating a plasmon. In a bulk material the plasmon quickly dissipates in heat as the electrons more around, and no effect is seen. Conduction electrons in a metal nanoparticle are not so free to move, they are confined in space. When light hits a metal nanoparticle, a plasmon is also generated by the surface conduction electrons, but this time the movement of the electrons is confined in space. Therefore *a surface localised plasmon* is generated. **The plasmon oscillates periodically in a confined space**. When the frequency of this oscillation is the same as the frequency of the light that it generated it (i.e., the incident light), the plasmon is said to be in resonance with the incident light. For this reason the effect is called the localised surface plasmon



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resonance effect (LSPR). **The consequence of the LSPR effect is that there is an enhanced electromagnetic field close to the surface/particle.**

The energy of LSPRs is **sensitive to the dielectric function of the material and the surroundings and to the shape and size of the nanoparticle**. This means that if a ligand, such as a protein, attaches to the surface of the metal nanoparticle, its LSPR energy changes. Similarly the LSPR effect is sensitive to other variations such as the distance between the nanoparticles, which can be changed by the presence of surfactants or ions. The LSPR effect has been observed not only on metal nanoparticles but also in nano-rings, voids in metal films and other nanostructures

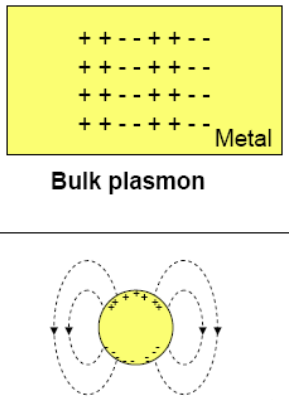


**Figure 2**. Formation of plasmons in bulk metal (top) and in nanoparticles (bottom). (Image credit: D. Sutherland, iNANO, Aarhus University, Creative commons Attribution ShareAlike 3.0)

One of the consequences of the LSPR effect in metal nanoparticles is that they have **very strong visible adsorption** due to the resonant coherent oscillation of the plasmons. As a result, colloids of metal nanoparticles such as gold or silver can display **colours which are not found in their bulk form**, like red, purple or orange, depending on the nanoparticles' shape, size and surrounding media.

**In this experiment you will see that nano-gold is not golden in colour but ruby-red!**

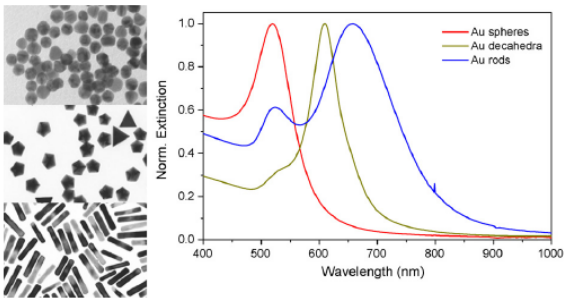


**Figure 3**. Transmission electron micrographs and UV/vis spectra of gold nanoparticle colloids with various geometries: (top) spheres, (middle) decahedra and (bottom) rods. (Image credit: Reprinted from: Borja Sepúlveda et al., "LSPR-based Nanobiosensors", Nano Today (2009), 4 (3), 244-251, with permission from Elsevier.



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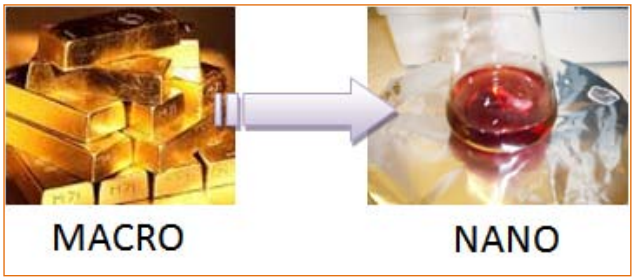


**Figure 4**. Dependence of colour on gold size. (Image credit: L. Filipponi, iNANO, Aarhus University, Creative Commons Attribution ShareAlike 3.0).

**Medieval artisans used it!** Without knowing it, artisans have been using nano-gold through history. The beautiful **stained glass windows produced during the medieval time**, and visible in numerous churches, are made of a composite of glass and nano-sized metal particles. The "ruby-red" glasses often seen are a mixture of glass with ultrafine (nanosized) gold powder.

## Does nano-gold have other properties different from bulk gold?

Yes, it does. Nano-gold is extremely reactive and is now being studied as a **new catalyst**. Nano-gold has been shown to be an extremely efficient catalyst in numerous pollution control studies. For example, a company has announced an engineered nano-gold oxidation catalyst which can reduce diesel hydrocarbon emission 40% times more than commercially available materials. Considering that there are over 14 million light-duty diesel vehicles worldwide, and 2 million heavy-duty ones, the impact of this nanotechnology could be enormous.

## What is the application of nano-gold in medicine?

Gold is now studied in many nanomedicine applications. Here we focus on one: its use as a colorimetric biosensor. Generally speaking, a *sensor* is a device capable of recognising one or more specific chemical species within a mixture and 'signalling' its presence through some chemical changes. A 'transducer' converts the chemical signal into a quantifiable signal with a defined sensitivity. A *biosensor* is a device that is capable of detecting a specific biomolecule, such as a type of antibody, a fragment of DNA, etc. The presence of these specific biomolecules is indicative of the presence of a certain type of virus or bacteria which is responsible for a specific disease. One area where research is very active is the development of future miniaturised biosensors that doctors can use in their office to test if their patient has a specific disease. This is called "point-of-care-diagnostics". Nanomaterials have most of their atoms


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on the surface and therefore have a large area (compared to their volume) available for detection. Also, as we will see in this experiment, nanomaterials have peculiar *optical properties* which make their use advantageous. For this and other reasons, nanomaterials are very useful for the engineering of miniaturised biosensors.

In a *gold colloidal plasmonic biosensor* the sensing event results in a **change of aggregation among the nanoparticles that form the colloid** (**Figure 5**).

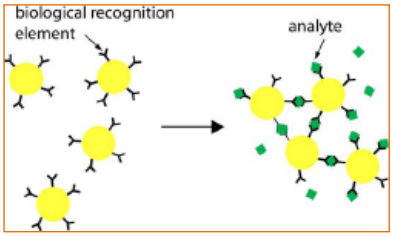


**Figure 5**. Schematic representation of a colloidal

This change of aggregation can determine a colour change of the colloid. Absorption spectroscopy is used to quantify the biosensing event. For this reason the sensor is called *colorimetric* (from the word "colour"). In the case of gold colloid, which is normally ruby-red, the sensing event can result in the colloid becoming blue. In nanomedicine this effect is used for instance in **genetic screening** where scientists look for a specific gene sequence in a sample. The gene sequence can be an indication of the presence of a specific pathogen, such as a virus. First, the sequence of bases in the target DNA is identified. Then two sets of gold particles are prepared — one has DNA attached that binds to one end of the target DNA, and the second set carries DNA that binds to the other end. The nanoparticles are dispersed in water. When the target DNA is added, it binds both types of nanoparticle together, linking them together to form an aggregate. The formation of this aggregate causes a shift in the light-scattering spectrum from the solution, that is, a colour change in the solution that can easily be detected. The example is illustrated in **Figure 6**.

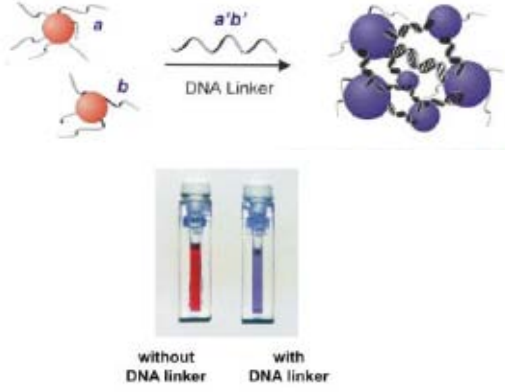


**Figure 6**. A plasmonic colloidal nanosensor. (Image credit: reprinted with permission from Jin et al., Journal of American Chemical Society (2003), 125 (6), 1643-54. Copyright 2003 American Chemical Society.

Other techniques use the key-and-lock mechanism of antigen-antibodies, or enzymes that can bind to specific biomolecules. Basically this technique combines the general concept of biorecognition (which is common to all biosensors) with a peculiar nano-effect (the fact that as the aggregation of gold nanoparticles changes, the colour changes).



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**In this experiment you will test a colloid gold nanosensor to see if it can detect an electrolyte, such as salt.**

You will perform the synthesis of a gold colloid starting from a solution of **gold chloride hydrate** and a **solution of sodium citrate**. This is the simplest reaction to synthesis gold nanoparticles and generally produces **gold nanoparticles 10-20nm in size**. In the reaction, the citrate acts as a weak reducing agent (reducing $AuCl^{4-}$ to Au) and as a stabiliser. A layer of citrate anions adsorbs around each nanoparticle and prevents these from aggregating: the anions' electrostatic repulsion keeps the nanoparticle separated. In this state, the colloid appears ruby-red owing to the absorption of light by the free electron oscillations (the surface plasmon). In this experiment $\lambda_{max}$ is around 520nm (green) and the solution appears red.

**- If the anion layer is removed, the nanoparticles start to approach and agglomerate.** This effect can be used for *sensing* a certain chemical. If a strong electrolyte is added, such as NaCl, the ions of the salt shield the negative charges on the particles, allowing them to approach and aggregate into larger and larger clumps. The formation of agglomerates is reflected in a change of the optical spectrum causing the solution to **turn deep blue**. If a high concentration of salt is added, the nanoparticles aggregate to a point at which they precipitate, and the solution becomes clear.

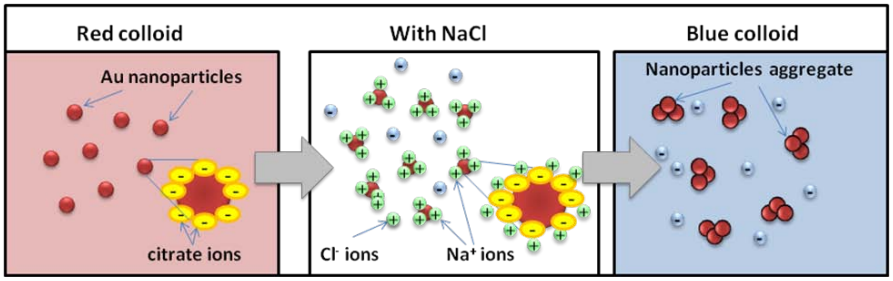


**Figure 7**. Schematic representation of the optical changes of a colloidal gold as a solution of salt is added. (Image credit: L. Filipponi, iNANO, Aarhus University, Creative Commons Attribution ShareAlike 3.0)



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**- If a weak or non-electrolyte is added** (e.g., sugar), the electrostatic repulsion between the gold and the citrate ions is not disrupted and the solution remains red.

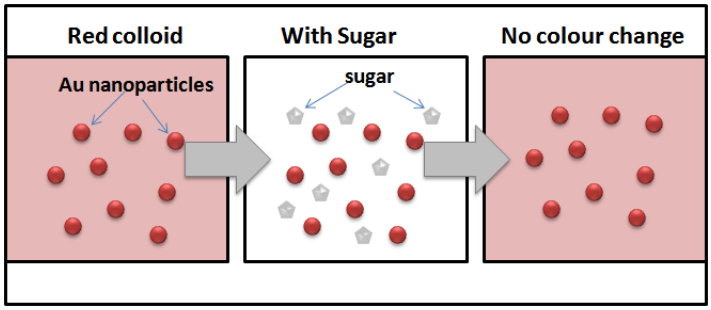


**Figure 8**. Schematic representation of the the optical changes of a colloidal gold as a solution of sugar is added. (Image credit: L. Filipponi, iNANO,

**- If a stabiliser of high molecular weight is added**, such as a protein or polyethylene glycol, it adsorbs to the surface of the nanoparticles with the effect of inhibiting aggregation, even at high salt concentration. In this exercise egg white is used as a very economic source of protein (mainly ovoalbumin).

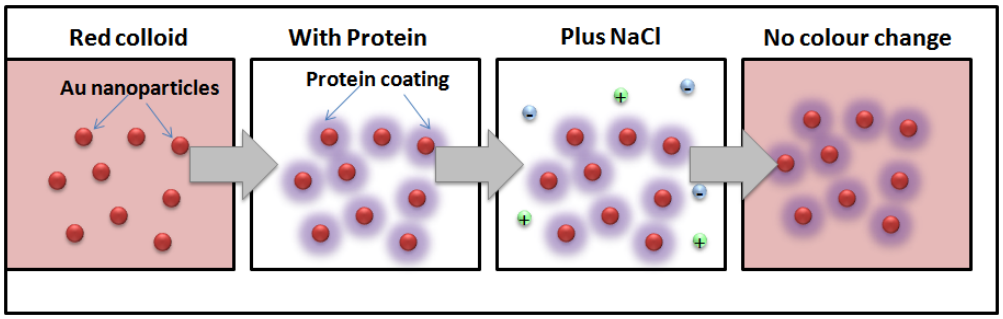


**Figure 9**. Schematic representation showing that a protein coats the gold nanoparticles and prevents them from aggregating as salt is added to the colloid. (Image credit: L. Filipponi, iNANO, Aarhus University, Creative Commons Attribution ShareAlike 3.0)

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**CREDIT**

This exercise was partly adapted from the experiment reported in: "Color my nanoworld", Journal of Chemical Education, Vol. 81(4), 2004 and from the experiment "Citrate synthesis of gold nanoparticles", University of Whisconsi-Madison, see: http://www.mrsec.wisc.edu/Edetc/curriculum/index.html; A more detailed description of the synthesis of colloid gold is given in: Keating et al., Journal of Chemical Education 1999, Vol. 76, No. 7 pp. 949-955.



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