Functionalization of Gold Nanoparticles for the instrument-free detection of Lead in water

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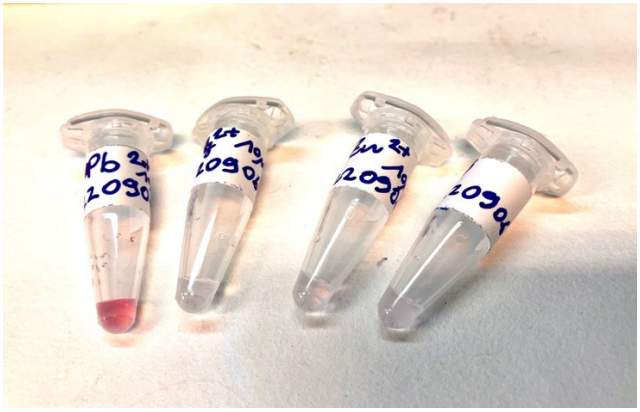
There are several pedagogical objectives in this practical. First, with the increasing interest for the use of short synthetic DNA strands in DNA-based nanotechnologies[1–3], you will explore some "un-natural" properties of DNA by immobilizing engineered DNA primers on the surface of gold nanoparticles for bio-sensing purposes[4–6]. Indeed, such applications rely on the tunable structure of short oligonucleotides for applications other than the "natural" genetic data-storage function of genomic DNA. For instance, only few years after aptamers have been described, a similar strategy has been described for the selection of DNA strands capable of RNA site-specific binding and cleavage[7]. As such biomolecules turned out to have similar activities as enzymes, they are named DNAzymes[8]. Programmable DNA self-assembly ability is also raising on increasing interest : If you wish to have more information and details on the use of DNA for nanotechnologies (DNA 2D and 3D nanostructures, DNA-based origamis or DNA nanowires), you are kindly invited to check the pioneer work[9] of Adrian Seeman, which has been followed by a steadily increasing number of contributors. Regarding aptamer-based sensing approaches, the competitive cost of oligonucleotides, their easy chemical functionalization and their high potential for small target (< 1,000 Da) detection -that could barely not be achieved with antibodies operated in immuno-assays-, is illustrated by the large number of emerging applications[10] and papers published every year, although commercial products based on aptamers are still missing[11].

Second, this practical involves the synthesis and use of nano-materials (gold 20 nm large nanoparticles) decorated with DNA probes bound to their surface. Such nanomaterial is easy to synthesize[12,13], cost effective, and quite safe. About this later point, although for any chemical reagent "the dose makes the poison", gold nanoparticle colloidal solutions have been used for centuries for putative curative properties[14]. Even nowadays, gold based nanoparticles are still discussed as potential therapeutic agents[15]. During this practical, you will use citrate-coated gold nanoparticles (AuNP) I synthesized myself by reduction of Au^{3+} ions in aqueous solutions[12]. This process is rather simple (one single step, and purification required as the process stops after consumption of the Au^{3+} ions initially present in solution), very cost-effective (total estimated cost for the reagents < 10 USD), and pretty fast (reaction completed within an hour). Such starting material is also commercially available and is quite affordable (about 100 USD for 25 mL of a nanomolar solution of 20-nm to 100-nm large gold nanoparticles). Although citrate-coated gold nanoparticles are quite stable in aqueous solutions, some spontaneous irreversible aggregation might be observed upon covalent modification. For this reason, the citrate ligand might be exchanged by a phosphine ligand[16]

(which turns out to be more expensive than the gold nanoparticle themselves!) before conjugation with the DNA strands.

On Day 1, you will use these phosphine-coated gold nanoparticles I synthesized in France and graft thiolated oligonucleotides on them by forming "self-assembled monolayers" (SAM). The process is quite fast (one hour), but it is followed by the AuNP surface saturation with another kind of thiolated molecules (PEG-SH) lasting 30 minutes, and then several centrifugation/resuspension steps ensuring the removal of any traces of unbound DNA. Then, on Day 2, the practical describes the operation of a assay[6,17], and the processing of the functionalized nanomaterial for the detection of the lead Pb^{2+} ions present in aqueous solutions. Similarly to other heavy metal ions, lead may contaminate rivers, water supply facilities and rain water tanks. This contamination may be due to mining activities, industrial accidental leakage (as lead is used in large amounts in batteries, gasoline refineries, and chemical industry (pigments and painting for instance). The World Health Organization (WHO) thus recommends level below 0.01 mg/Liter in drinking water (source: WHO/SDE/WSH/03.04/09 reference document), corresponding to more or less 50 nM of Pb^{2+} ions. In the USA, the maximum contamination level of Pb^{2+} in drinking water is to be at most 72 nM, (source: United States Environmental Protection Agency -EPA). In this practical, you will take benefit of the spontaneous catalytic activity of DNA strands enabling the cleavage of a nucleic acid substrate strand in presence of Pb^{2+} ions. The selective process (SELEX) operated for the identification of such DNA strand capable of alternative strand cleavage has been carried in presence of lead Pb^{2+} ions[18], and confirmed a highly selective, and dependent, process, as no reaction occur in absence of lead Pb^{2+} ions nor in presence of other metallic cationic ions. Practical attendees will be taught to prepare a sample range to assess both the sensor range ability and its limit of detection.



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