COMBINED METHODS IN MAXIMIZING DNA LOADING ON TO GOLD NANOPARTICLES

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An enormous amount of effort is being put into the development of effective ways of conjugating biomolecules on to the surface of nanoparticles (NP). Our design of NP - organic molecule conjugates pretends to control both properties of the inorganic core and those of the coating molecules. Conjugation refers to the attachment achieved by a chemical reaction between function groups of the inorganic nanomaterial and those of the biomolecules.

For biomedicine the nanomaterial used must be biocompatible and nontoxic, for which colloid gold is generally preferred to synthesize small spherical-like particles. These are then functionalized with the different biomolecules, which could be proteins, such as enzymes or antibodies; sequences of nucleotides, such as DNA or RNA; lipids and sugars, or a combination of these molecules. Possible applications range from simple analytical tools to complicated drug delivery, control of the immune system, and use in therapies. In the case of nucleotids, the characteristic base pairing could be used in diagnostic in the detection of specific sequences and mutations[1]; in determining DNA binding proteins and intercalators; control of gene expression using antisense RNA[2]; DNA scaffolds[3]; amongst others.

The conjugation of DNA to gold nanoparticles is mediated by a thiol group synthetically added on to the 5'end of the single strand sequence. This is the commonly known way of achieving conjugation to gold surfaces. However conjugation of DNA is not as straightforward as simply adding these modified strands to a nanoparticle solution.

Following the work of A.Paul Alivisatos and Chad A. Mirkin on DNA loading onto gold nanoparticles, our procedure is a combination of the two, the main objective being to maximize the coverage of the particles with single strand DNA and to follow the loading process using agarose gel electrophoresis.

The conjugation of nucleotides to AuNPs is specially challenging due to the high affinity of the phosphate DNA backbone for the gold surface which compete with the thiol groups leading to an undesirable wrapping of the NP with the DNA strands, rather than the intended radial conjugation (see figure 1A).

By combining experimental procedures we are optimizing the process as everything is done to minimize non-specific interactions between the DNA strand and the gold surface, by coating with Bis(p-sulfonatophenyl) phenylphosphine dihydrate dipotassium salt, or to break these interactions if they are formed. Ultimately NaCl is used to stretch the strands and allows further conjugation to the exposed surface.

DNA is added once at the beginning and salt is added gradually. After each salt addition, aliquots are taken and characterized by UV-VIS spectrophotometer and -potential to study the increased loading on the surface and the stretching of the strands which increase the particle size. These aliquots are analyzed in agarose gel electrophoresis where

visualization of the progressive coverage is achieved and appears as less migration in the gel every time the salt concentration is increased (see figure 1B).

The kinetics of the loading, followed by a simple electrophoresis where no staining is needed thanks to the presence of high optically active AuNPs, could have straight forward applications in determining interaction of molecules to DNA, and how, and to what extent, they interact affecting the functionalization process. Our hypothesis is that the interaction of DNA binding molecules should modify the process in a simple and reproducible manner.

This work is a presentation of the different procedures used in the synthesis of monodisperse particles of the required size, their functionalization with single strand DNA using a combined method of the different pre-existing experimental procedures, and completed with a selection of experiments using DNA binding molecules.

Amongst the DNA binding molecules tested is cisplatin, a platinum-based chemotherapy drug that works cross-linking DNA strands, used to treat various types of cancers, such as sarcomas, some carcinomas, lymphomas and germ cell tumours.

The project is also a preliminary study in understanding control of the number of strands conjugated, as achieved by Alivisatos, to develop DNA probes.

References:

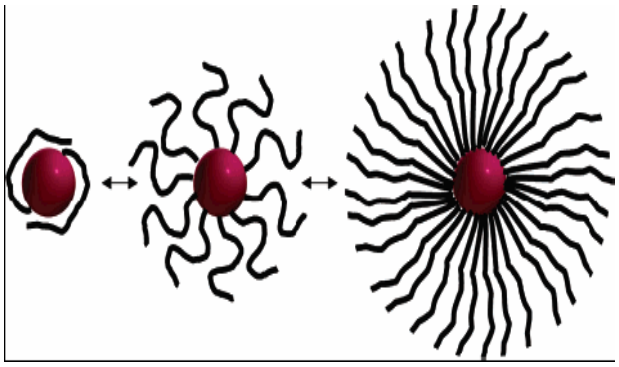
[1] Gerion,D.; Chen, F.; Kannan, B; Fu,A.; Parak, W.J.; Chen, D.J.; Majumdar, A.; Alivisatos, A. P.; Anal. Chem. 2003, 75.

[2] Rosi, N.L.; Giljohann, D.A.; Thaxton, C.S.; Lytton-Jean, A.K.R.; Min Su Han; Mirkin,C.A.; SCIENCE, vol 312, 2006.

[3] Zanchet, D.; Micheel, C.M.; Parak, W.J.; Gerion,D; Williams, S.C.; Alivisatos, A.P.; J. Phys. Chem B., 2002, 106

Figures:

A) **B)**



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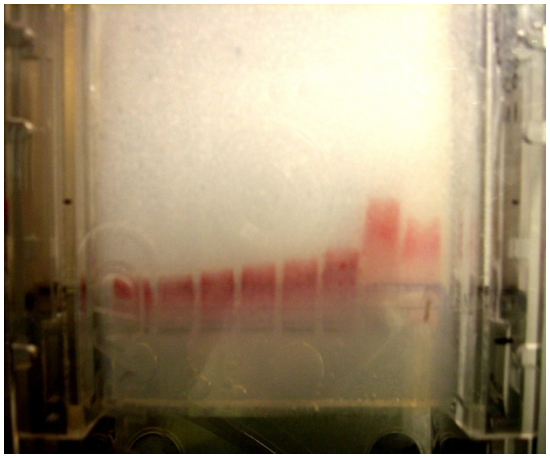

Fig.1. A) The DNA loading protocol minimizes the undesired interactions that stop further conjugation. Avoiding DNA wrapping around the nanoparticle is critical when wanting to maximize coverage. B) From right to left, each aliquot run in the gel (3% agarose) represents an increase in salt concentration during the conjugation process, consequently they migrate less due to an increase in size.