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PASSIVE ADSORPTION OF PROTEINS

The conjugation of proteins to gold nanoparticles could be performed via conventional adsorption or via covalent conjugation. In order to perform the conventional adsorption strategy classical citrate capped gold nanoparticles are employed. This strategy is simpler when the pH of gold nanoparticles is close to the isoelectric point of the protein of interest.

Bioconjugates obtained by passive adsorption are stabilized through different phenomena: electrostatic interaction between negative charged nanoparticles and the positive charged positions in the protein, the hydrophobic interaction between protein and gold surface, and the strong affinity between the metal and the conducting electrons of nitrogen and sulphur atoms in the protein structure.

CONJUGATION OF PROTEINS TO METALLIC NANOPARTICLES (Passive Adsorption)

Applicable to

Citrate capped gold nanoparticles, tannic acid capped silver nanoparticles and citrate capped gold/silver alloy nanoparticles.

Materials

Citrated capped metallic nanoparticles

10% (w/v) NaCl

2 mM sodium citrate

0.1 % (w/v) Tween 20

UV-Vis spectrophotometer

1XPBS (Phosphate Buffered Saline).

OPTIMIZATION: pH and protein concentration.

The aim of this stage is to estimate the minimum amount of protein required to stabilize the citrate capped metallic nanoparticles. Despite the fact that the optimum pH to perform the adsorption of proteins to the nanoparticle is close

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to the isoelectric point of the protein, we recommend you to optimize the pH value in a small range.

For each condition to be tested:

1. Transfer 200 µL of citrate capped nanoparticles into 1.5 mL microcentrifuge tube.
2. Centrifuge your sample at a suitable centrifugation force (*See Table 1*).
3. Remove the supernatant, and resuspend your sample in 2 mM sodium citrate at the desired pH.
4. Then add between 0 and 50 µg of protein in 20 µL to your metallic nanoparticles.
5. Mix well for 30 minutes making use of a rotary shaker at room temperature.
6. Add 200 µL of 10% (w/v) NaCl and incubate again for 30 minutes at room temperature.
7. If there is not enough amount of protein (nanoparticle surface is not saturated) nanoparticles will aggregate in presence of NaCl, and a color change will be observed by naked eye (e. g. from red to blue for gold nanoparticles, and from yellow to grey for silver nanoparticles) in the solution due to nanoparticle destabilization.

To perform a more accurate optimization, it is recommended employing an UV-spectrophotometer as a detection system. Nanoparticle aggregation could be measured as an increase in absorbance at 650 nm (gold nanoparticles) or 690 nm (silver or gold silver alloyed nanoparticles).

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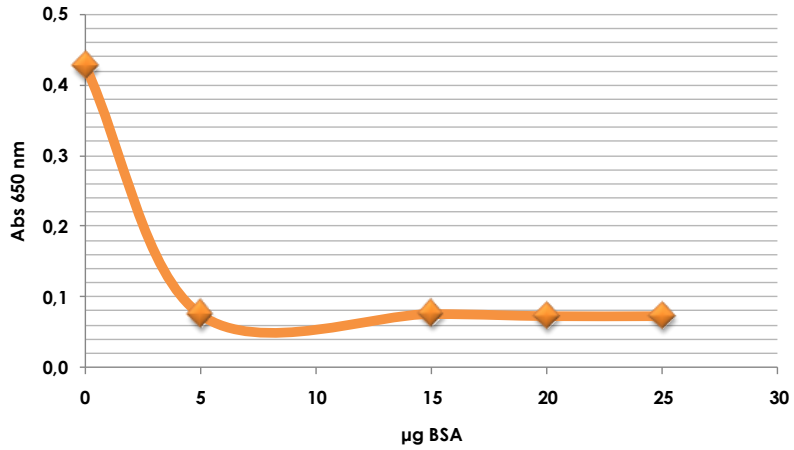

Example of the variation of the absorbance at 650 nm from suspensions of AuNP 20 nm incubated with different amount of BSA after NaCl addition.

CONJUGATION PROTOCOL:

1. Transfer your selected amount of nanoparticles to a microtube.
2. Centrifuge your sample in presence of Tween 20 (final concentration 0.025% w/v).
3. Remove the supernatant and resuspend the nanoparticles in sodium citrate 2 mM to the original volume at the optimized pH value.
4. Add the amount of protein calculated previously plus an additional 10%.
5. Incubate for 60 minutes on a rotary shaker.
6. Purify the solution by centrifugation at an adequate speed.
7. Resuspend your bioconjugate in PBSx1 BSA 1%.
8. Validate the functionality of your conjugate.
9. Store your conjugate at 4ºC. Do not freeze.

Sample volume: 1 mL	
Time: 30 min	
Diameter (nm)	Speed (g)
15	15000
20	7500
30	4500
40	2500
50	2000
60	1125
70	600
90	500
100	400

Table 1