PREPARATION OF PROTEIN A GOLD

Paul Webster
Center for Cell Imaging, Yale School of Medicine

Colloidal gold has been used for centuries in the preparation of stained glass for windows and fine glassware. In recent years, colloidal gold particles have become a useful tool in microscopy for staining tissues and sections. Colloidal gold particles are especially useful for biological electron microscopy. Some of the reasons why are listed below.

- Homogeneous preparations of particles varying in size from 3 µm to 20 µm can be easily prepared.
- Colloidal gold suspensions are inexpensive to prepare. Most proteins can be easily coupled to colloidal gold particles.
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- Proteins coupled to gold particles do not appear to lose their biological activity.
- The colloidal gold particles can be easily seen in the electron microscope.
- Colloidal gold does not naturally occur in biological material. Therefore, if you see it, it is because you put it there.
- Colloidal gold probes can be used for light microscopy. The larger gold particles can be directly observed by the light microscope. Small particles are detected by silver enhancement or epipolarized illumination.
- The same probes can be used for both LM and TEM imrnunocytochemistry.

Here I explain a simple, published protocol for preparing colloidal gold particles, how to couple these particles to protein A, and how to purify the probes after they have been made.

This article is based on, and liberally borrows from the following two published articles by Jan W. Slot and Hans J. Geuze:

J.W. Slot & H.J. Geuze 1981. Sizing of protein A-colliodal gold probes for immunoelectron microscopy. J.Cell Biol. **90,** 533-536.

J.W. Slot & H.J. Geuze 1985. A new method of preparing gold probes for multiple-labeling cytochemistry. Europ. J.Cell Biol. 38, 87-93.

PREPARATION PROCEDURE FOR PRODUCING GOLD SOLS

To make 100 ml of gold sol:

Solution A) 80 ml H_20+ 1 ml 1 % gold chloride

Solution B) 4 ml 1% tri-sodium citrate.$2H_20$ + 16 ml H_20 + variable amount of 1% tannic acid [Mallinckrodt #1764] (Table 1).

When 1 ml or more tannic acid is needed, add an equal amount of 25mM potassium carbonate for pH adjustment.

Warm up solutions A and B to 60°C and mix them while stirring. When the red colour has formed, heat up to 95°C and cool the solution on ice. The larger particles (where lower concentrations of tannic acid are used) take longer to form and the red colour can take up to 1 hr to develop.

COUPLING THE PROTEIN A TO THE GOLD PARTICLES

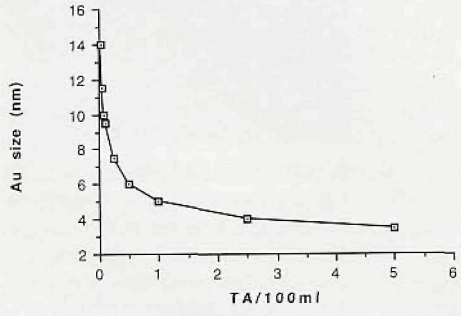
A gold sol will bind proteins more efficiently when the pH of the solution is close to the pI of the protein (for protein A the pI is pH 5.1). A tighter binding occurs at higher pH but this may have a denaturing effect on the protein making the probe less effective. Having too much protein coupled to the gold particles may also be disadvantageous: some of the weakly bound protein may detach from the particles. This will make the probe less effective because the free protein will compete for binding sites with the gold-labelled protein. Horrisberger and Clerc (1985, Labelling of colloidal gold with protein A. A quantitative study. Histochemistry, **82**, 219-223) recommend binding the protein A to colloidal gold at pH 6.0.

Check the pH of the gold sol with pH paper (the gold sol will block the electrode on the pH meter) and adjust the pH with 0. 1N NaOH.

Protein A is dissolved in distilled water at 1 mg/ml.

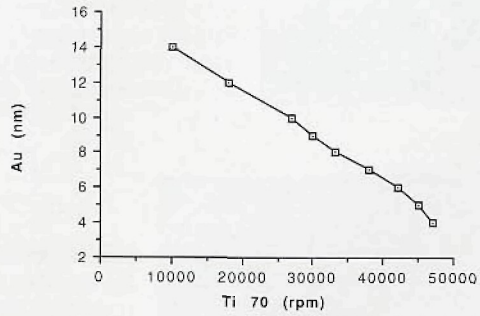
A microtitration assay will show the correct amount of protein A to add to the gold sol (between 4-6 µg/ml). If only small amounts of protein are available to be coupled to the colloidal gold then the titration assay can be performed in micro amounts. The degree of aggregation, instead of being assessed using color changes, can be determined in the TEM. Small amounts of the assay mix are adsorbed onto coated specimen grids, dried and examined in the TEM. The correct amount of stabilization will result in single gold particles being adsorbed onto the film. Too little protein and the gold particles will be aggregated.

Add the protein A while stirring the gold sol. After 5 min add 10% bovine serum albumin (BSA) in PBS to a final concentration of 0.2% (2 ml/l00 ml) to maximally stabilize the sol.



Au (nm)	1% Tannic acid /100 ml (ml)
3.5	5.000
4.0	2.500
5.0	1.000
6.0	0.500
7.5	0.250
9.5	0.100
10.0	0.080
11.5	0.050
14.0	0.025

Table 1: The influence of the tannic acid concentration, during gold sol formation, an the size of the gold particles. [Add equal mount of 25 mM potassium carbonate (K_2CO_2) when 1 ml or more of tannic acid is used]



Au (nm)	Ti 70 (rpm)	g (r_{max})
4	47000	225000
5	45000	210000
6	42000	185000
7	38000	155000
8	33000	105000
9	30000	92000
10	27000	75000
12	18000	37000
14	10000	7000

Table 2: First contrifugation: Beckman Centrifuge, Ti 70 rotor for 30 min at 4°C.

PURIFICATION OF THE PROTEIN A GOLD

The protein A gold is centrifuged in a Ti 70 rotor in a Beckman ultracentrifuge at the appropriate speed (Table 2) for 30 mm at 4°C.

At the correct speed the gold particles will settle to the bottom of the tube as a loose pellet. Remove the supernatant without disturbing the pellet and resuspend the loose part of the pellet in PBS containing 0.2% BSA.

A second centrifugation step down a gradient will remove any gold particles of the wrong size. This is done on a 10-30% continuous glycerol gradient at 4°C.

Layer 1-2 ml of protein A gold onto the top of the gradient, spin in an SW40 rotor at the appropriate speed (see table 3) for 45 min at 4°C. The dark red band in the middle of the gradient is collected. All aggregated protein A gold particles will have been removed.

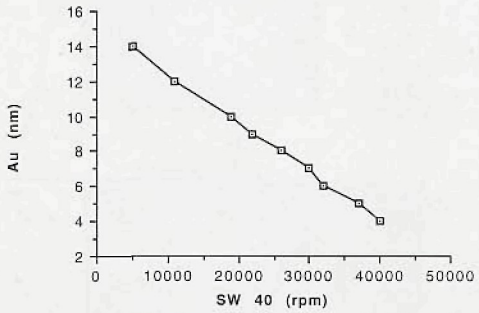
STORAGE AND USE

Immunogold probes may lose activity within weeks, due to the dissociation of the proteins from the gold particles. Many users suggest using only freshly prepared colloidal gold probes, others recommend storing frozen in cryoprotectant. There is little documented evidence to allow for comparisons to be made. However, if the gold probes are to be stored frozen, dialyse the protein - gold against 50% glycerol in PBS and store at -20°C or freeze down small aliquots in liquid nitrogen and store at -70°C.

To determine the concentration at which to use the protein A gold, measure the optical density at 520 nm of a 1:100 diluted solution in PBS. Use a dilution with an O.D. of between 0.05 and 0.1 where there is no significant background. If using a primary antibody then the optimal dilution of this first antibody must be known. Sections can be treated with protein A gold alone to determine the backgroung labelling.

COUPLING OTHER PROTEINS TO GOLD PARTICLES

Using the rule of coupling proteins to gold sol at a pH similar to the pI of the protein, it is possible to couple virtually any protein to gold. Transferrin-gold and bovine serum albumin (BSA)-gold have been successfully used in many studies on endocytosis, enzymes have been coupled to gold and used to locate their substrates, receptors and ligands and many different antibodies have been coupled to gold. (See "Griffiths, G. 1993. Fine Structure Immunocytochemistry. Springer Verlag", for more detailed examples of the use of colloidal gold probes in biomedical research.)



ɹ (nm)	SW 40 (rpm)	g (r_{max})	
	40000	284000	1 hr
	37000	240000	or 1hr at 35K
	32000	170000	
	30000	150000	
	26000	115000	
	22000	85000	
)	19000	65000	
2	11000	20000	
4	5000	5000	

Table 3: Second centrifugation: Beckman Centrifuge, SW 40 rotor, 45 min 4° C,

BSA-gold is a useful marker for studying the endocytic processes in mammalian cells. For most experiments large amounts of the gold probe suspension are required. In this instance, preparing the probe in the laboratory is a cost-effective way of obtaining the large quantities required. Typically, the living cells are incubated in a BSA-gold suspension with a final OD, at 520 nm, of 4. The above protocol for preparing protein A-gold can be followed for preparing BSA-gold but BSA is substituted for the protein A. Usually, the stabilization requires more BSA than protein A (we use 24 µg/ml). The centrifugation values are the same.

After the gold probe has been concentrated and purified it is dyalized against PBS, or culture medium, before being added to the living cells.

If proteins other than protein A or BSA are to be coupled, then the centrifugations speeds must be adjusted to take into account the different masses of the proteins. For example, apo-transferrin-gold will centrifuge at the same speeds as shown for protein A-gold but diferric transferrin required slower speeds. The colloidal gold coupled to high density and low density lipoproteins will also require slower centrifugation speeds.

PROTEIN A GOLD SUPPLY

In addition to the commercial suppliers (Amersham, Goldmark, Polysciences etc.), it is possible to arrange with Jan Slot to supply protein A-gold probes. This laboratory is not a commercial supplier but will provide fresh preparations of protein A-gold throughout the year. We buy 1 ml. of protein A-gold which is supplied to us in four 250 µl installments (one every three months). It is sent as soon as it is made, so there are no questionable shelf life problems and the suppliers also use the gold for their own research, which acts as an ongoing quality control.

For more details, contact:
Jan Slot or George Postuma
University of Utrecht,
Department of Cell Biology,
Medical School, AZU HOZ.314,
Heidelberglaan 100,
3584 CX Utrecht,
The Netherlands.
Fax: 0113130541797
eMail: j.w.slot@lab.azu.nl