Advanced Techniques in Biology and Medicine

Nekouian, et al., Adv Tech Biol Med 2014, 2:1
http://dx.doi.org/10.4172/2379-1764.1000118

Research Article Open Access

Development of Gold Nanoparticle Based Colorimetric Biosensor for Detection of Fibronectin in Lung Cancer Cell Line

Reza Nekouian[*1], **Najme Javdani Khalife**[2], **Zahra Salehi**[2]

[1]*Department of Biotechnology, School of Allied Medicine, Iran University of Medical Sciences (IUMS), Fardis lab, Iran*
[2]*Cellular and Molecular Research Center (CMRC), Iran University of Medical Sciences (IUMS), Tehran, Iran*

[*]**Corresponding author:** Reza Nekouian, Department of Biotechnology, School of Allied Medicine, Iran University of Medical Sciences (IUMS), Fardis lab, Iran, Tel: 98-9125161759; Fax: 98-21-88622533; E-mail: reza_nik@unipune.ernet.in

Rec date: May 08 2014, **Acc date:** May 20 2014, **Pub date:** Jul 05 2014

Abstract

Specific protein detection by means of antibody-nanoparticle conjugates is a new field in medical nanobiotechnology. Among many nanoparticles used, gold nanoparticles show strong light-absorption properties which have been exploited in designing nanobiosensors. Fibronectin (FN) plays an important role in extracellular matrix (ECM) structure and function of normal cells; however, in conditions like lung carcinoma its expression increases, especially in non-small cell lung carcinoma (NSCLC). In this study, we conjugated gold nanoparticles to human fibronectin antibody (anti-hFN) to design a colorimetric nanobiosensor for detection of FN present in ECM of cultured cells. Three different cell lines namely A549 (target cells), AGO-1522 (control cells) and Nalm-6 (negative control cells) were used to compare changes in color resulting from aggregation of gold nanoparticles due to higher amount of FN. Our construct was able to detect increased level of FN which was distinguishable visually by change in color and could be confirmed by spectrophotometer as well.

Keywords: Fibronectin (FN); Gold nanoparticles (GNPs); Lung carcinoma; Nanobiosensor.

Introduction

Broad applications of nanomaterials in the field of biology and medicine helped scientists to find a way to change many clinical and traditional diagnostic and prognostic methods [1,2]. Cancer nanotechnology is a new area of medical nanobiotechnology research which has a great impact on detection and diagnosis of cancer cells [1-3]. While accurate detection of cancer cells with the help of nanobiotechnology has various obstacles, optical detection paves a new road for rapid and reliable method in this area [3,4]. Numerous colorimetric nanobiosensors with specifically designed nanoparticles have the potential to detect specific cell types within the target organs [5]. To design colorimetric nanobiosensors, some metal nanoparticles (<100 nm) might be used to exhibit strong shift in colors due to their surface plasmon resonance (SPR) peak in the visible range [6,7]. Gold nanoparticles (AuNPs) are used for their specific characteristics like biofunctionalization, biostability, spectral properties and surface plasmon resonance peak which occur in the visible range depending upon their size and shape [8]. AuNPs exhibit prominent distance-dependent optical properties which reveals when they come close to each other, their absorption and scattering properties are altered which leads to a change in color and shift in absorption spectra [9,10]. Strong light-absorption properties of AuNPs is a distinguished feature which has been used in designing biosensors for diagnostic and therapeutic approaches and many techniques have been emerged considering these properties which all are based on aggregation of gold nanoparticles to identify target molecules [11-14]. However, conjugation of AuNPs to monoclonal antibodies with high affinity makes them a precious biomarker to detect the target protein in cancer cells [15].

Among the most abundant extracellular matrix components such as collagens, tenascins, proteoglycans, glycosaminoglycans and laminin, fibronectin plays an important role both in ECM structure and function [16,17]. Fibronectin is a high-molecular-weight adhesive glycoproteins which is composed of two large monomers (250 kDa) that are linked by disulfide bonds at their carboxy terminal ends [18,19]. After lung injury, the expression of cellular fibronectin is increased mainly due to promotion of fibroblasts proliferation [20,21]. Fibronectin has been found expressed more in lung cancer, specifically in non-small cell lung carcinoma (NSCLC). Similar studies on small cell lung carcinoma (SCLC) showed higher levels of fibronectin in all adult tissue samples as well [22].

In our study, by conjugating AuNPs to human fibronectin antibody (anti-hFN) a colorimetric biosensor has been designed to detect fibronectin (FN) which might be present in extracellular matrix (ECM) of human cultured cells. Aggregation of anti-human fibronectin gold nanoparticles (anti-hFN-AuNPs) is based on targeting fibronectin through its recognition in the ECM of the cultured cells. Overlapping surface plasmon resonance of AuNPs due to their assembly in the ECM, leads to a shift in absorption spectra and alteration in light scattering as well which causes signal transduction and change in color. In this study, our target cells was A549 lung cancer cells whereas cultured human skin (AGO-1522) and hematopoietic cell line (Nalm-6) were used as control and negative control cells respectively.

Materials and Methods

Gold nanoparticle preparation

For preparation of gold nanoparticles all glassware were cleaned in aqua regia (3 parts HCL, 1 part HNO3). Distilled and deionized water used for the preparation of all solutions. Gold nanoparticles (AuNPs)

with a diameter of ~30 nm were prepared by citrate reduction of Gold III chloride hydrate (HAuCl4) which purchased from sigma based on the method introduced by Turkevich [23]. Based on this method, 50 ml of HAuCl4 1M (2.5×10-4) was prepared. Then, 0.5 ml of 34 mM citrate was added to boiling HAuCl4 solution. The synthesis of AuNPs was monitored by observing changes in the absorption band based on surface plasmon resonance of AuNPs.

Cell culture

Three different cell lines namely human Caucasian lung carcinoma type 2 epithelial cells (A549), human skin (AGO-1522) and hematopoietic (Nalm-6) were used as target, control and negative control cells respectively and all were obtained from National Cell Bank of Iran (NCBI). The cells were cultured in DMEM medium (Dulbecco's Modified Eagle Medium) with 10% FBS (Fetal Bovine Serum) and 1% penicillin-streptomycin, all purchased from Biosera. Cells were incubated at 37°C in an atmosphere of CO2/air (5/95%) saturated in water. Counting of cells was performed to keep the cell number constant throughout the procedure.

UV–Vis spectroscopy

Optical extinction spectra were recorded using digital UV-visible spectrophotometer (Ultrospect 4300pro) in the 300-800 nm range and the graphs were analyzed using SWIFTII software.

Fabrication of anti-hFN-AuNPs conjugate

Monoclonal anti human fibronectin (F0791) was purchased from Sigma Aldrich. To fabricate anti-hFN-AuNPs conjugate, coagulation test was carried out ahead in order to find out the optimal concentration of antibody solution which minimizes the disturbance of the nanoparticle-antibody complex. 25 µl of 30 nm AuNPs were mixed well with 50 µg/100 µl of anti-hFN. Conjugation of AuNPs with anti-hFN antibody was performed in room temperature (RT) and the pH was adjusted to 7-9. The mixture was incubated for 5 minutes and then to remove the excess antibody the solution was centrifuged at 14000 rpm for 20 minutes at 4°C and resuspended in 20mM HEPES storage buffer (pH=7.4-7.5) for further use. The prepared anti-hFN-AuNPs conjugate was stored at 4-8°C. Fabrication of anti-hFN-AuNPs was based on non-covalent interactions (electrostatic coupling).

Detection of fibronectin in cellular matrix by anti-hFN-AuNPs complex

For antibody-nanoparticle (Anti-hFN-AuNPs) treatment constant number of the cells (100000 cells) were grown in 12-well tissue culture test-plates (from Orange Scientific Company) and counted before adding anti-hFN-AuNPs and analysis of aggregation process. After 72 hours of cell culture, the medium was initially removed gently and then washed with buffer (HEPES 20 mM). Then 500 µl of the anti-hFN-AuNPs conjugate was added to each well containing approximately 100000 cells and the plate was gently shaken and incubated for 30 minutes.

Results and Discussion

Construction of anti-hFN-AuNPs complex and detection of fibronectin

A monoclonal anti human fibronectin (anti-hFN) was conjugated to 30 nm gold nanoparticles (AuNPs) to confirm selectivity, specificity and colorimetric aspects of the experiment. In figure 1 the spectra of (a) and (b) which are gold nanoparticle and gold nanoparticle conjugated to anti human fibronectin at 530 nm and 700 nm respectively show almost complete conjugation state of nanoparticle and antibody complex based on the report by Smita Thobhani and colleagues [24]. The color of two solutions (a) and (b) was pinkish (Figure 1. B. a). The constructed nanoparticle-antibody was used to examine its recognition and binding to the fibronectin present in the ECM of cultured target cells (A549) (Figure 1. A. c). Comparison between two spectra (b) and (c) shows a drastic change at 530 and 700 nm which confirms alteration in absorption and scattering of gold nanoparticles (Figure 1. A. b and c) and a prominent visual change in color from pink to violet (Figure 1. B. a and b). A schematic representation (Figure 2) is designed based on our preliminary data which tries to explain the whole scenario.

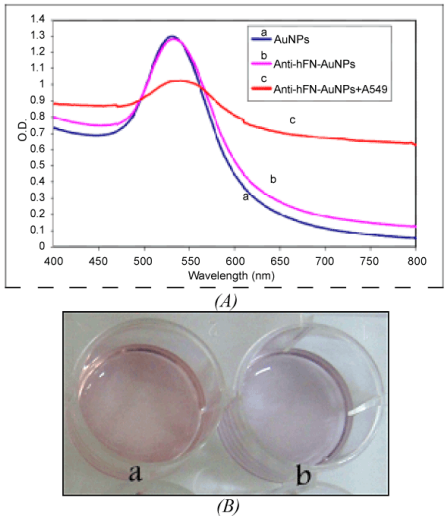

Figure 1 A: Spectra of the gold nanoparticle aggregation in presence of fibronectin in target cells: (a) AuNPs, (b) conjugated AuNPs with anti human fibronectin and (c) conjugated AuNPs with anti human fibronectin in presence of (A549). B: Visual comparison of changing color in two wells. (a) The well containing anti-hFN-AuNPs with no cell (blank) and (b) the well containing anti-hFN-AuNPs with target cells (A549).

The spectrum of anti-hFN-AuNPs (a) denotes higher range of absorbance comparing to the spectra of anti-hFN-AuNPs with target cells (c) which is due to the binding of anti-hFN-AuNPs to the fibronectin present in the ECM of the target cells. 100 µl of NaCl 1 M was added to the samples to determine binding of antibody-gold nanoparticle complex to the fibronectin of the ECM of cultured cells, as in absence of fibronectin NaCl disturbs the ion charge balance of the solution due to the most aggregated condition of AuNPs (b), whereas anti-hFN-AuNPs (a) without NaCl shows the highest pick absorbance. The pick spectrum of anti-hFN-AuNPs+A549+NaCl (d)

was very close to anti-hFN-AuNPs+A549 (c) even after addition of NaCl which indicates almost complete and selective binding and relatively the most aggregated condition in the solution when the target cells are present. Based on the results obtained from spectra of different samples, it was clear that Anti-hFN-AuNPs was bound selectively to fibronectin present in the ECM of the cells and gathering of the Anti-hFN-AuNPs in the matrix of the target cells leads to alteration in the absorption and scattering of the gold nanoparticles. NaCl is mostly used as a negative confirmatory test as it disrupts the electric change of the AuNPs and it causes the most aggregated state of the AuNPs, the large-scale assembly of the particles and change in their spectral properties. Comparison of two spectra, anti-hFN-AuNPs +target cells and anti-hFN-AuNPs+target cells+NaCl were relatively no different mainly due to selective binding of the anti-hFN-AuNPs to the fibronectin, aggregation of particles and ultimately elimination of the possibility of any more aggregation by NaCl.

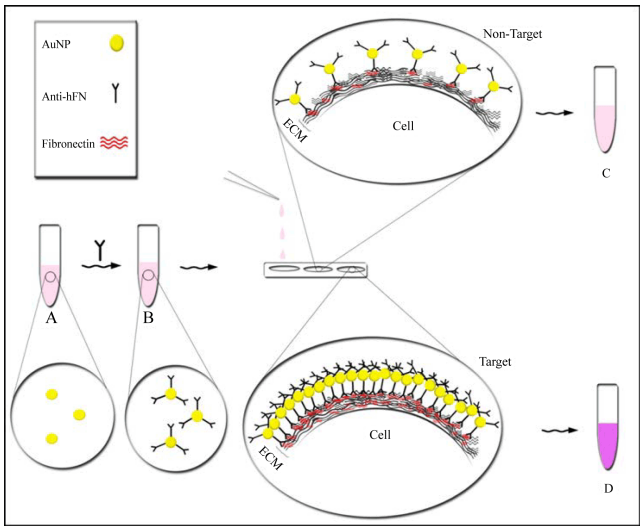

Figure 2: Schematic illustration of antibody-nanoparticle complex with target and non target cells: Steps involved in conjugation of gold nanopartcles with anti human fibronectin are shown in A and B. Aggregation and assembly of the antibody-nanoparticle complex in target and non target (control) cells are shown in C and D.

Examining constructed antibody-nanoparticle complex by NaCl

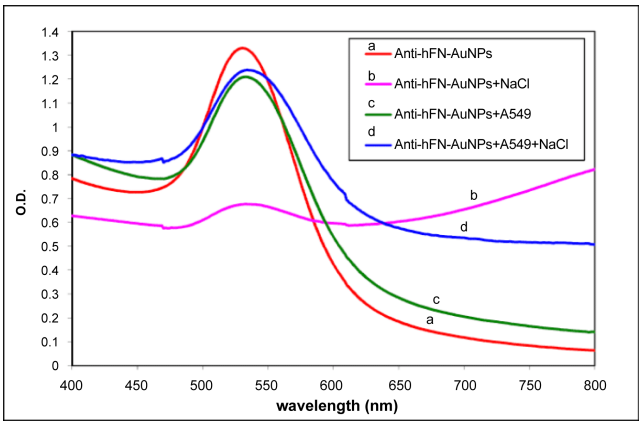

Figure 3: Comparison of four different samples to confirm binding of constructed antibody-nanoparticle complex to the target cells (A549): (a and b) show human fibronectin antibody-gold nanoparticle complex and human fibronectin antibody-gold nanoparticle + NaCl 1 M respectively. (c and d) show human fibronectin antibody-gold nanoparticle in presence of target cells and human fibronectin antibody-gold nanoparticle in presence of target cells + NaCl 1 M respectively.

Usage of antibody-nanoparticle complex with target and non target cell lines

As fibronectin generally is present in the ECM of the most attached cell lines, binding of the Anti-hFN-AuNPs to these attached cell line was expected but it was important to differentiate between ECMs with much higher amounts of fibronectin.

Figure 4 shows the results of using Anti-hFN-AuNPs with different cell lines. Spectrum (a) is anti-hFN-AuNPs solution without any cell with the plasmon resonance peak at 530 nm with the absorbance of 1.4. However, a closer look in the spectra b, c and d shows decrease in the level of absorbance at 530-550 nm. The assembly and selectively embedding of the Anti-hFN-AuNPs in and around the ECM of the AGO-1522 and A549 cell lines due to the present of fibronectin leads to alteration in the light scattering and absorption properties of the gold nanoparticles. To be realistic, it is difficult to reach definite selectivity regardless of the molecular marker used, and nonselective binding to the suspended cells devoid of ECM and fibronectin may occur which is highly unlikely to be to an extant to assure false positive results.

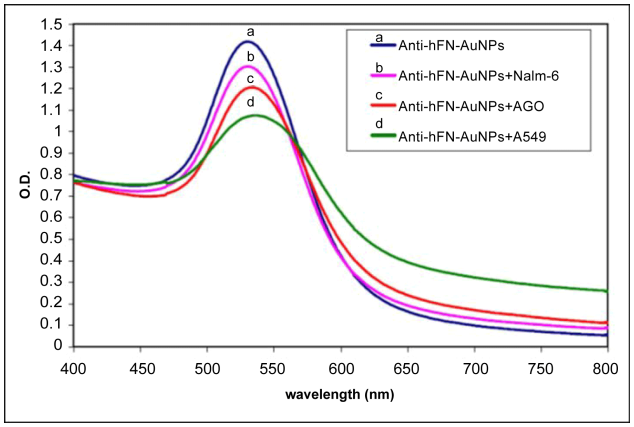

Figure 4: Spectra of nano conjugate with target and non target cell lines: (a) Anti-hFN-AuNPs (no cell), (b) Anti-hFN-AuNPs + Nalm-6 (negative control cell line), (c) Anti-hFN-AuNPs + AGO-1522 (control cell line), (d) Anti-hFN-AuNPs + A549 (target cell line).

However, in figure 4 comparing the spectra of the target cells (A549) (d), control and negative control cells (AGO-1522 (c) and Nalm-6 (b)) the absorbance was observed in the long wavenumber region over 600 to 750 nm (Figure 4. b, c and d) and towards the low-energy of the absorption spectrum area, near IR. High absorption is achieved with the target cell line (A549) containing larger particle aggregates which are due to higher level of fibronectin in its ECM.

It is noteworthy to mention that A549 cell line, adenocarcinomic human alveolar basal epithelial cell line is known to express fibronectin in higher amounts. A review article by Jeffrey D. Ritzenthaler et al. in 2008 clearly discussed about alteration of fibronectin expression in lung carcinoma and higher levels of fibronectin in both non small cell lung carcinoma (NSCLC) and small cell lung carcinomas (SCLC) [18].

Incubation time

The effect of incubation time of anti-hFN-AuNPs with the target cells on the rate of aggregation was studied and the results demonstrated that aggregation of anti-hFN-AuNPs takes about 60 minutes to complete (data not shown). However there was a change in color from pink to violet within 30 minutes. At longer times there was no change in color beyond what was seen in 30 minutes.

Effect of cell number

The effect of increase in cell number on aggregation rate and level of optical density (O.D.) at the wavelength of 530 nm is shown in figure 5. The results showed that when the number of cells is below 100000, O.D. is relatively cell number dependent. However, when the number of cells increases (above 100000 cells) the rate of aggregation and consequently O.D. is independent of cell number and increase in cell number does not change the rate of aggregation. We also found that the maximum O.D. did not change as the number of cells was increased beyond certain limits. In fact, increase in cell number leads to accumulation of more fibronectin in ECM of cells but the amount of anti-hFN-AuNPs complex added was consistent in each well. Although the designed biosensor is quantitative in nature, color differences in samples with variable cell numbers were not distinguishable enough by naked eye especially in high cell numbers. Additionally, the major purpose of this study was to design a convenient colorimetric biosensor by which one can detect overexpression of fibronectin in a lung cancer cell line without specialized equipment by naked eye.

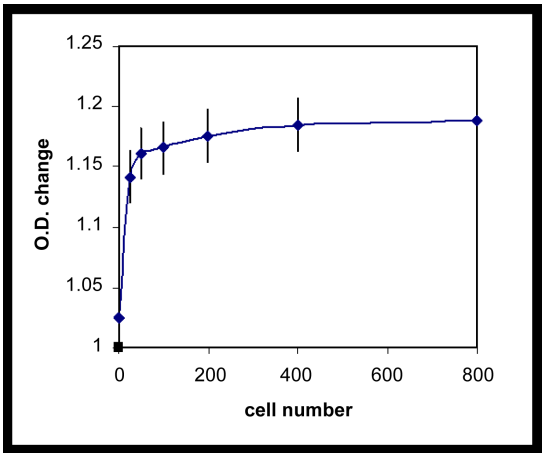

Figure 5: The cell number effect: The cell number in wells of the plates were designed to contain from 0 (no cell) to 800000 cells. O.D. changes are measured at 530 nm wavelength.

Conclusion

Detection of cancer cells with the help of a technique without any invasive procedure and sophisticated instruments is an ideal goal in diagnosis of cancer. In our preliminary study, we constructed a complex containing gold nanoparticles conjugated with anti-human fibronectin (Anti-hFN-AuNPs) to bind specifically to fibronectin of ECM of cultured cells.

Anti-hFN-AuNPs complex selectively bond to fibronectin of the cells containing ECM, however ECM of the cells with higher levels of fibronectin showed more prominent alteration in absorption and scattering due to more aggregation of gold nanoparticles. Based on the preliminary results we conclude that this constructed complex has the ability to detect cancer cells with higher level of fibronectin like lung cancer visually, just by change in color.

Acknowledgements

This work was funded by Cellular and Molecular Research Center (CMRC), Iran university of medical sciences, Tehran, Iran.

References

1. Giljohann DA, Seferos DS, Daniel WL, Massich MD, Patel PC, et al. (2010) Gold nanoparticles for biology and medicine. Angew Chem Int Ed Engl 49: 3280-3294.
2. Heath JR, Davis ME (2008) Nanotechnology and cancer. Annu Rev Med 59: 251-265.
3. Zhu X, Cao Y, Liang Z, Li G (2010) Aptamer-based and DNAzyme-linked colorimetric detection of cancer cells. Protein Cell 1: 842-846.

4. Wang X, Wu L, Ren J, Miyoshi D, Sugimoto N, et al. (2011) Label-free colorimetric and quantitative detection of cancer marker protein using noncrosslinking aggregation of Au/Ag nanoparticles induced by target-specific peptide probe. Biosens Bioelectron 26: 4804-4809.
5. Kim CS, Wilder-Smith P, Ahn YC, Liaw LH, Chen Z, et al. (2009) Enhanced detection of early-stage oral cancer in vivo by optical coherence tomography using multimodal delivery of gold nanoparticles. J Biomed Opt 14: 034008.
6. Kalele SA, Kundu AA, Gosavi SW, Deobagkar DN, Deobagkar DD, et al. (2006) Rapid detection of Escherichia coli by using antibody-conjugated silver nanoshells. Small 2: 335-338.
7. Schofield CL, Haines AH, Field RA, Russell DA (2006) Silver and gold glyconanoparticles for colorimetric bioassays. Langmuir 22: 6707-6711.
8. Haiss W, Thanh NT, Aveyard J, Fernig DG (2007) Determination of size and concentration of gold nanoparticles from UV-vis spectra. Anal Chem 79: 4215-4221.
9. Reynolds RA, Mirkin CA, Letsinger RL (2000) Homogeneous, nanoparticle-based quantitative colorimetric detection of oligonucleotides. J Am Chem Soc 122: 3795–3796.
10. Sandrock ML, Foss CA (1999) Synthesis and linear optical properties of nanoscopic gold particle pair structures. The Journal of Physical Chemistry 103: 11398-11406.
11. Choi JW, Kang DY, Jang YH, Kim HH, Min J, et al. (2008) Ultra-sensitive surface plasmon resonance based immunosensor for prostate-specific antigen using gold nanoparticle–antibody complex. Colloids and Surfaces 313-314: 655–659.
12. Elghanian R, Storhoff JJ, Mucic RC, Letsinger RL, Mirkin CA (1997) Selective colorimetric detection of polynucleotides based on the distance-dependent optical properties of gold nanoparticles. Science 277: 1078-1081.
13. Medley CD, Smith JE, Tang Z, Wu Y, Bamrungsap S, et al. (2008) Gold nanoparticle-based colorimetric assay for the direct detection of cancerous cells. Anal Chem 80: 1067-1072.
14. Thanh NT, Rosenzweig Z (2002) Development of an aggregation-based immunoassay for anti-protein A using gold nanoparticles. Anal Chem 74: 1624-1628.
15. Dykman LA, Bogatyrev VA, Khlebtsov BN, Khlebtsov NG (2005) A protein assay based on colloidal gold conjugates with trypsin. Anal Biochem 341: 16-21.
16. Johansson S, Svineng G, Wennerberg K, Armulik A, Lohikangas L (1997) Fibronectin-integrin interactions. Front Biosci 2: d126-146.
17. Ruoslahti E (1988) Fibronectin and its receptors. Annu Rev Biochem 57: 375-413.
18. Ritzenthaler JD, Han S, Roman J (2008) Stimulation of lung carcinoma cell growth by fibronectin-integrin signalling. Mol Biosyst 4: 1160-1169.
19. Peters JH, Trevithick JE, Johnson P, Hynes RO (1995) Expression of the alternatively spliced EIIIB segment of fibronectin. Cell Adhes Commun 3: 67-89.
20. Limper AH, Roman J (1992) Fibronectin. A versatile matrix protein with roles in thoracic development, repair and infection. Chest 101: 1663-1673.
21. Roman J (1997) Fibronectin and fibronectin receptors in lung development. Exp Lung Res 23: 147-159.
22. Buttery RC, Rintoul RC, Sethi T (2004) Small cell lung cancer: the importance of the extracellular matrix. Int J Biochem Cell Biol 36: 1154-1160.
23. Turkevich J (1985) Colloidal gold. Part 1. Gold Bull 18: 86-91.
24. Thobhani S, Attree S, Boyd R, Kumarswami N, Noble J, et al. (2010) Bioconjugation and characterisation of gold colloid-labelled proteins. J Immunol Methods 356: 60-69.