### SUPPORTING INFORMATION FOR:

### Method for the quantitative extraction of gold nanoparticles from human bronchoalveolar lavage fluids through a glycerol gradient

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Supporting File S1: Derivation of formula for the extent of interdiffusion; calculation of the diffusion coefficient; relationships between glycerol concentration and solution viscosity and density; Nernst equation for the calculation of reduction potential; calculation of effective particle density.

### Formula for the extent of interdiffusion d

To derive the formula for the extent of interdiffusion d inside the cushion at any given time t, it was assumed that at the fringes of diffusion, glycerol concentration (C) needed to be  $\geq 97.5\%$  of its initial value C<sub>gc</sub>. Under the described experimental conditions, the relation between the initial glycerol concentrations in the aliquot (C<sub>al</sub>) and the cushion (C<sub>gc</sub>) in Step II is C<sub>gc</sub> = 5 C<sub>al</sub> (due to glycerol carried over from Step I). Substituting these conditions on the solution to Fick's second law for interdiffusing liquids

 $\begin{array}{l} C\ (\ x,\ t\ )=0.5\ (\ C_{gc}+C_{al}\ )-0.5\ (C_{gc}-C_{al})\ erf\ (\ 0.5\ x\ D^{-1/2}\ t^{-1/2})\ ,\\ &\ it\ becomes\\ 0.975\ C_{gc}=0.5\ (\ C_{gc}+C_{gc}\ /\ 5\ )-0.5\ (C_{gc}-C_{gc}\ /\ 5)\ erf\ (\ 0.5\ d\ D^{-1/2}\ t^{-1/2}\ )\\ &\ and\ eventually\\ -\ erf\ (\ 0.5\ d\ D^{-1/2}\ t^{-1/2}\ )=\ 1.317\\ &\ or\\ d=2.634\ \ D^{1/2}\ t^{1/2}\approx\ (\ 7\ D\ t\ )\ ^{1/2} \end{array}$ 

#### Calculation of the diffusion coefficient D

The diffusion coefficient D for Step II was calculated by observing the interdiffusion between 0.5 ml of cushion and a mixture of 1ml BWF with 0.25 ml cushion (to represent the amount of cushion carried over from Step I, as calculated by eq. 2), over the course of t = 4 h at 0°C. The extent of interdiffusion was the distance between the initial and final position of the interface as evaluated by the grayscale color profile of the smeared interface in ImageJ (Figure A). Assuming that d = (7 D t)<sup>1/2</sup>, it was calculated that D ≈ 4.4 10<sup>-11</sup> m<sup>2</sup> s<sup>-1</sup>.

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Relationship between solution density and glycerol concentration in glycerol-water solutions

The density  $\rho$  of glycerol-water solutions at 0°C at various glycerol concentrations was calculated based on a linear regression extrapolated by data found in the Handbook of Chemistry and Physics [1], presented in Figure B.



# Relationship between solution viscosity and glycerol concentration in glycerol-water solutions

Viscosity  $\eta$  was calculated according to the formula proposed by Cheng [2]:

 $\eta = \exp(alpha*log(0.00173))*exp((1-alpha)*log(10.693))$ 

where

 $alpha = (1-C_mass) + (\alpha^*\beta^*C_mass^*(1-C_mass)) / (\alpha^*C_mass + \beta^*(1-C_mass)),$ 

C\_mass is the mass fraction of glycerol, and

α, β are temperature-based parameters, with  $\alpha$  = 0.705 and β = 2.045 for T = 0°C.

Nernst equation for the reduction potential of blank BWF

 $E_{CIO^{-}} = E^{0}_{CIO^{-}} - (RT / n_{e}F) ln([CI^{-}] / [CIO^{-}])$ 

### where

 $E_{CIO^{-}}$  is the half-cell oxidation potential of CIO<sup>-</sup>,

E<sup>0</sup> CIO<sup>-</sup> is the standard half-cell oxidation potential of CIO<sup>-</sup> in an alkaline solution,

R is the universal gas constant (8.3 J K<sup>-1</sup> mol<sup>-1</sup>),

T is the temperature in kelvin,

F is the Faraday constant (9.6 10<sup>4</sup> C mol<sup>-1</sup>),

ne is the number of moles of electrons transferred in the half-cell reaction, and

[CI-], [CIO-] are the molar concentrations of CI- and CIO- according to the nominal concentrations of saline and NaOCI solutions.

### Effective density of AuNP

The effective density  $\rho_{ED}$  of suspended AuNP in BWF was roughly estimated as 17.055 g cm<sup>-3</sup> using the Sterling equation [3]:

$$ED = (1 - \varepsilon_{\alpha}) \rho_{p} + \varepsilon_{\alpha} \rho_{BWF}$$

where  $\rho_p$  is the primary particle nominal density (19.3 g cm<sup>-3</sup>),

 $\rho_{BWF}$  is the BWF density, numerically equal to its relative density (1.094 g cm<sup>-3</sup>), and  $\epsilon_{\alpha}$  is the AuNP agglomerate porosity:

$$\epsilon_{\alpha} = 1 - (d_H d_{P^{-1}})^{DF - 3}$$

where d<sub>H</sub> is the AuNP agglomerate Z-average size measured by DLS (74.8 nm), d<sub>P</sub> is the smallest primary particle diameter observed by TEM (45 nm), and DF is a theoretical fractal dimension, equal to 2.3. Since it was impossible to calculate the agglomerate Z-average size in BWF, its value in DPBS was used instead. Also, the Sterling equation requires the particle diameter as determined by the Brunauer Emmet Teller method, but since AuNP were directly prepared in suspension, their primary particle size by TEM was used instead. Finally, the DF value was the one suggested by Deloid et al. for AuNP in cell culture media [4]. The estimated  $\rho_{ED}$  was used in conjunction with the smallest observed primary particle diameter d<sub>P</sub> (45nm) so that the model returns the most conservative values regarding the required duration of the centrifugation in Step II.

1. Haynes WM: CRC handbook of chemistry and physics. CRC press; 2014.

2. Cheng N-S: Formula for the viscosity of a glycerol–water mixture. Industrial & engineering chemistry research 2008, 47:3285-3288.

3. Cohen JM, Teeguarden JG, Demokritou P: An integrated approach for the in vitro dosimetry of engineered nanomaterials. Part Fibre Toxicol 2014, 11:20.

4. DeLoid G, Cohen JM, Darrah T, Derk R, Rojanasakul L, Pyrgiotakis G, Wohlleben W, Demokritou P: Estimating the effective density of engineered nanomaterials for in vitro dosimetry. Nat Commun 2014, 5:3514.

## Supporting Figure S2: Sample preparation for field emission scanning electron microscopy (FESEM) and back-scatter electron microscopy (BSE)

**A**, **B**) A mica sheet is deposited on the aluminium stub where it can be secured with a small piece of double-sided adhesive tape.

**C)** The stub was then placed in a Gatan 682 precision coating system and a 50 nm high-resolution carbon layer was deposited on top of the mica sheet.

**D)** The coated mica sheet was electrically connected to the second rim of the stub with conductive tape.

**E)** Sample drops (1-2  $\mu$ I) were deposited on the coated surface and were left to dry with the stub positioned horizontally and protected by environmental air.



#### Supporting Table S3: Dissolution of nanoparticles in blank BWF

The reduction potential and pH of blank BWF (50% v/v of 0.0183 M Na-hypochlorite in 0.9 w/v NaCl) were calculated at 0.87 V and 11.4, respectively. These values are expected to become lower in the presence of biomolecules found in real BWFs. The compositional stability of several prominent nanoparticles was assessed in blank BWF. Specifically, citrate-capped Au and Ag, Al<sub>2</sub>O<sub>3</sub>, Fe, amorphous SiO<sub>2</sub>, TiO<sub>2</sub>, and ZnO nanoparticle suspensions at varied concentrations were added to 1.00 ml of blank BWF in 2ml Protein LoBind tubes (Eppendorf<sup>®</sup>) and vortexed for a few seconds. The tubes were then sealed and stored at 4°C for a minimum of 1 week, protected from light. To measure the dissolved particle fraction, 0.40 ml of each dispersion was transferred in 100 kDa Amicon<sup>®</sup> Ultra-0.5 centrifugal filter tubes of a nominal molecular weight cut-off of 100 kDa (Merck Millipore<sup>™</sup>) and centrifuged at 14000×g in a fixed-angle rotor (Eppendorf<sup>®</sup> MiniSpin Plus<sup>™</sup> centrifuge, ThermoFisher Scientific<sup>™</sup>) for 10 min at room temperature. The filtrates (≈0.4 ml) were then added in 9.60 ml of 2.0 M HCl and the concentration of dissolved metals were measured by ICP-OES against a a standard multi-element solution in 5% w/w HNO<sub>3</sub> (SCP Science). Results indicated that Al<sub>2</sub>O<sub>3</sub>, citrate-capped Au, Fe, TiO<sub>2</sub>, and ZnO were minimally or not dissolved at all; citrate-capped Aq nanoparticles were completely dissolved, most probably by the formation of AgCl; amorphous SiO<sub>2</sub> were almost completely dissolved (71%).

prominent nanoparticles.								
core composition	Ag	Al <sub>2</sub> O <sub>3</sub>	Au	Fe	SiO <sub>2</sub>	TiO <sub>2</sub>	ZnO	
size range (nm) *nominal	36-44	30-60	61-73	35-45	22*	≤150*	≤100 *	

Table 3	<b>33</b> Primary	particle	size	and	dissolution	in	blank	BWF	of	а	panel	of
promin	ent nanopai	rticles.										

% dissolution 106.3 0.6 0.0 71.0 6.0 nonnon-S.D. 1.9 0.4 detectable 0.0 detectable 1.0 0.0

### Supporting Figure S4: Application of the extraction method on fetal bovine serum spiked with AuNP

The extraction method was performed on undiluted fetal bovine serum spiked with AuNP at 2.50, 5.00, 7.50, and 10.00% of the stock particle concentration. In this medium, protein molecules adsorb on the particles' surface but have been shown to cause only low agglomeration [1]. Extraction yields were similar to those achieved in BWF, except for the highest concentration where less agglomeration in FBS allowed for even better yields. AuNP were spread throughout the cushion and allowed for the plasmon resonance effect to take place (image below). Some pelleting did still occur at the highest particle concentration, but to less extent when compared to BWF (inset of image below). The extraction yield as measured by ICP-OES ranged from 70 to 86% attaining similar levels to BWF, but performing significantly better at the highest concentration.



1. Casals E, Pfaller T, Duschl A, Oostingh GJ, Puntes V: Time evolution of the nanoparticle protein corona. *ACS Nano* 2010, 4:3623-3632.

## Supporting File S5: Application of the extraction method on bronchial washing fluids from two patients

The method was applied on bronchial washing fluids from two patients (A and B) with symptoms of infiltrative pulmonary diseases. A summary of their clinical data is presented in Table 1; ICP-OES and DLS data are summarized in Figures A and B, respectively; optical microscopy, BSE, and in-lens FESEM images along with particle perimeter analysis by ImageJ are presented in Figure C. Despite the presented data, a full mineralogical analysis of the extracted would require additional experiments, e.g. high-resolution EDX and TEM measurements in order to determine the chemical composition and crystal structure of the visualised particles

Table 1 Clinical data summary collected from patients A and B

	Tobacco use	Residential environment	Professional exposure	Nanoparticle exposure	Symptoms & findings	Diagnosis
A	Former	Rural	Asbestos (15 years)	Perfume Hydration creme	Acute CT scan: lung shadow	Drug-induced lung disease
в	Non- smoker	Rural	-	Perfume Hydration creme Toothpaste	Chronic CT scan: micronodule	Lymphangitis carcinomatosa



Metal (Al, Co, Cr, Cu, Fe, Ni, Ti, W, Zn, Zr) concentrations (Figure A) and dkcps signals (Figure B) of the nanoparticle-containing fraction of patient B were generally stronger than the respective signals retrieved from patient A, possibly indicating a larger nanoparticle loads.



Figure C

i, ii) Optical microscopy could only resolve coarse particles extracted after Step I.

**iii, iv)** BSE images of smaller particles extracted after Step II: this technique detects electronically dense and/or protruding particles; dashed white lines are visual aids along the borders of the dried sample droplets where particles have been deposited due to the coffee ring effect effect. Interestingly, very large particles (measuring several microns in perimeter) are largely absent, indicating that they were successfully extracted in Step I.

**v**, **vi**) Higher magnification, in-lens FESEM imaging is very sensitive to morphological changes of the examined surface and revealed a markedly denser submicron and nanosized particle population for patient B.

Scale-bars: i, ii, 50 µm; iii, iv, 10 µm; v, vi, 2 µm.

#### particle size distributions:

Coarse particle populations presented similar size distributions under optical microscopy, but patient B carried more than twice particles than patient A.

BSE imaging detected mainly submicron-sized particles in patient B while very few particles (12) were detected in patient A. Finally, Under in-lens FESEM imaging, patient B carried ×6 timed more nanoparticles than patient A.

### Supporting File S6: Calculation of organic shell thickness and composition by XPS

In a core-shell system consisting of a spherical nanoparticle with a thin nanometric overlayer, the thickness of the shell can be calculated using the intensities ratio of photoelectrons coming from the shell and photoelectrons coming from the core, weighted by intensities of pure shell and core materials [1]. In our case, this ratio gives the following equation:

$$A = \frac{I_x I_{Au4f}^{\infty}}{I_{Au4f} I_x^{\infty}} \qquad \text{eq. 1}$$

where *x* is an element constituting the organic shell (C, O or N). The method takes also into account the travelling path of photoelectrons with two parameters, *B* and *C*, corresponding to ratios of attenuation length  $L_{a,b}$  of photoelectrons coming from core level a and travelling through material b. The two ratios are defined as:

$$B = \frac{L_{Au4f,shell}}{L_{x,shell}}$$
eq. 2
$$C = \frac{L_{Au4f,shell}}{L_{Au4f,core}}$$
eq. 3

The values of  $L_{a,b}$ , which are reported in Table 1, were estimated according to equation S4 from the paper of Seah [2] and as demonstrated in the work of Belsey et al. [3]. According to Shard, the shell thickness  $T_{NP}$  for a nanoparticle with a core radius R can be obtained with a precision of 4% using the following set of equations :

$$T_{NP} = \frac{T_{R\sim 1} + \beta T_0}{1 + \beta} \qquad \text{eq. 4}$$

$$T_0 = R\left[\left(ABC + 1\right)^{1/3} - 1\right]$$
 eq. 5

$$T_{R \sim 1} = \frac{T_{R \rightarrow \infty} R}{R + \alpha}$$
 eq. 6

$$\alpha = \frac{1.8}{A^{0.1}B^{0.5}C^{0.4}}$$
 eq. 7

$$\beta = \frac{0.13\alpha^{2.5}}{R^{1.5}}$$
 eq. 8

$$T_{R \to \infty} = \frac{0.74A^{3.6}\ln(A)B^{-0.9} + 4.2AB^{-0.41}}{A^{3.6} + 8.9}$$
 eq. 9

It is worth noting that, R and  $T_{NP}$  are expressed in units of  $L_{Au4f,shell}$ .

Because intensities from pure organic shell material cannot be obtained, equation 10 from the work of Belsey et al. was used, where [x] and [Au] are the atomic percentage of the elements x and Au considering that these elements are homogeneously distributed in all the depth of analysis,  $c_x$  the atomic fraction of the element x in the organic shell, and *f* an additional factor used to compensate for the different attenuation length, density, and intrinsic loss processes between the gold core and organic shell:

$$A_x = f \frac{[x]}{[Au]c_x}$$
 eq. 10

In this work, we make the assumption that the value of *f* is close to the one from the work of Belsey et al., which gives 0.56. As described in their work, a shell thickness  $T_{NP}$  is calculated for each element *x* present in the shell. With the constraint that the sum of all  $c_x$  equals 1, the result is computed iteratively varying the value of  $c_x$  until the same  $T_{NP}$  is found for each ratio  $A_x$ .

The computation of  $T_{NP}$  was carried out considering an averaged core radius of 30nm for the nanoparticles. Oxygen and carbon were considered as the elements forming the organic shell in the case of stock gold nanoparticles while oxygen, carbon and nitrogen were considered for the extracted gold nanoparticles. The calculation was made using the intensities of the photoelectron peaks O1s, C1s and N1s for the shell while for the core signal the Au4f peak was chosen.

For the O1s peak, the intensity of the component Si-O assigned to the silicon wafer native oxide was excluded and subtracted from the total intensity of O1s. For the C1s peak, in the case of stock AuNP, the intensity of the component assigned to adventitious carbon on the silicon wafer was also excluded. Using these assumptions, the atomic fraction of oxygen and carbon calculated for the organic layer on the stock nanoparticles with the iterative procedure were estimated respectively to 0.53 and 0.47 which is in accordance to the chemical formula of the citrate molecule. For the extracted nanoparticles, it is not possible to distinguish the adventitious carbon from the C-C and/or C-H chemical bindings in the proteins molecules. However, since we consider that the adventitious carbon comes mainly from the area of silicon wafer not covered by AuNP, we attempt to find an expression of the intensity of adventitious carbon for the sample with extracted nanoparticles

 $I_{C1s}^{advC/extractedNF}$ 

advC/stockNP

in function of the one for the sample with stock nanoparticles  $I_{Cls}^{\mu\mu\nu}$ Equation 11 shows that the ratio of the intensity of Si2p for a silicon reference sample  $I_{Si2p}^{ref}$  and for the sample with stock AuNP can be written as a linear relationship with 1/ $\cos\theta$  where  $\theta$  is the escape angle of the photoelectrons with respect to the normal of the sample. This expression allows estimating the thickness of the adventitious carbon layer dadvC/stockNP and the fraction of the wafer not covered by the nanoparticles  $\sigma^{advC/stockNP}$ . The same type of relationship can be written for the ratio of the intensity of Si2p for the sample with stock AuNP and the sample with extracted AuNP as shown in equation 12. This second expression allows calculating the adventitious carbon layer dadvC/stockNP and the fraction of the wafer not covered by the nanoparticles  $\sigma^{advC/stockNP}$  for the sample with extracted AuNP.

$$\frac{d^{advC/stockNP}}{L_{Si2p,advC}\cos\theta} + \ln(\sigma^{advC/stockNP}) = \ln\left(\frac{I_{Si2p}^{ref}}{I_{Si2p}^{stockNP}}\right)$$
eq. 11
$$\frac{d^{advC/extractedNP} - d^{advC/stockNP}}{L_{Si2p,advC}\cos\theta} - \ln\left(\frac{\sigma^{advC/extractedNP}}{\sigma^{advC/stockNP}}\right) = \ln\left(\frac{I_{Si2p}^{stockNP}}{I_{Si2p}^{extractedNP}}\right)$$
eq. 12

Figure C presents the adjustment of these linear equations with XPS measurements at different photoelectron takeoff angles. The fitting results allows for the calculation of the thicknesses and coverages of the adventitious carbon layer for both stock and extracted AuNP, using the attenuation length in Table 1 for L<sub>Si2p,advC</sub> which are presented in Table 2. The intensity of the adventitious carbon component in the C1s peak for the sample with extracted AuNP can be described by the following equation:

$$I_{C1s}^{advC/extractedNP} = I_{C1s}^{advC/stockNP} \times \frac{\sigma^{advC/extractedNP}}{\sigma^{advC/stockNP}} \times \frac{\left[1 - \exp\left(\frac{-d^{advC/extractedNP}}{L_{C1s,advC}\cos\theta}\right)\right]}{\left[1 - \exp\left(\frac{-d^{advC/stockNP}}{L_{C1s,advC}\cos\theta}\right)\right]}$$
eq. 13

Using the values in Table 2, the attenuation length L<sub>C1s,advC</sub> given in Table 1 and for a takeoff angle  $\theta$  of 50°, this intensity can be written as:

$$I_{C1s}^{advC/extractedNP} = 1.151 \times I_{C1s}^{advC/stockNP}$$
eq. 14

This intensity was then subtracted from the intensity of the C1s peak in order to calculate the thickness and composition of the organic shell for the extracted AuNP.

**Table 1** Attenuation length calculated using the equation S4 in the paper of for electrons coming from different core levels and travelling through a system consisting of gold nanoparticle core with an organic shell.

	L (organic shell), nm	L (Au core), nm
C1s	3.32	1.17
O1s	2.74	0.99
N1s	3.05	1.09
Au4f	3.79	1.33
Si2p	3.75	~

**Figure C** Adjustment of the expression given by equations 11 and 12 with experimental XPS measurements at several photoelectrons collection angles of the intensity of Si2p peak for a reference sample, the sample with stock Au nanoparticles and the sample with extracted Au nanoparticles.



**Table 2** Thicknesses and coverage of the adventitious carbon layer on the siliconwafer substrate for the sample with stock and extracted nanoparticles.

sample	$d^{\scriptscriptstyle advC}$ (nm)	$\sigma^{advC}$
Si/stock Au nanoparticles	1.36	0.78
Si/extracted Au nanoparticles	1.85	0.73

1. Shard AG: A straightforward method for interpreting XPS data from core–shell nanoparticles. The Journal of Physical Chemistry C 2012, 116:16806-16813.

2. Seah M: Simple universal curve for the energy-dependent electron attenuation length for all materials. Surface and Interface Analysis 2012, 44:1353-1359.

3. Belsey NA, Shard AG, Minelli C: Analysis of protein coatings on gold nanoparticles by XPS and liquid-based particle sizing techniques. Biointerphases 2015, 10:019012.