ARTICLE

DOI: 10.1038/s42004-018-0054-7

OPEN

Direct quantification of nanoparticle surface hydrophobicity

Andrea Valsesia¹, Cloé Desmet^{1,2}, Isaac Ojea-Jiménez¹, Arianna Oddo^{1,3}, Robin Capomaccio¹, François Rossi^{1,4} & Pascal Colpo¹

Hydrophobicity is an important parameter for the risk assessment of chemicals, but standardised quantitative methods for the determination of hydrophobicity cannot be applied to nanomaterials. Here we describe a method for the direct quantification of the surface energy and hydrophobicity of nanomaterials. The quantification is obtained by comparing the nanomaterial binding affinity to two or more engineered collectors, i.e. surfaces with tuned hydrophobicity. In order to validate the concept, the method is applied to a set of nanoparticles with varying degrees of hydrophobicity. The technique described represents an alternative to the use of other methods such as hydrophobic interaction chromatography or water-octanol partition, which provide only qualitative values of hydrophobicity.

¹European Commission, Joint Research Centre (JRC), Directorate F - Health, Consumers and Reference Materials, Via E. Fermi 2749, 21027 Ispra, VA, Italy.

²Present address: Université Grenoble Alpes, CEA, CNRS, INAC, SyMMES, Grenoble F-38000, France. ³Present address: Drug Delivery, Disposition and Dynamics, Monash Institute of Pharmaceutical Sciences, Monash University, Royal Parade 381, Parkville 3052, Australia. ⁴Present address: European Commission, Joint Research Centre (JRC), Directorate G - Nuclear Safety and Security, Westerduinweg 3, Petten NL-1755, The Netherlands. Correspondence and requests for materials should be addressed to A.V. (email: andrea.valsesia@ec.europa.eu)

Engineered nanomaterials (NMs) are widely used in many consumer and healthcare products, as well as novel nanomedicines.¹ To enable a quick and reliable NMs risk-benefit assessment, there is an urgent need for robust, standardised and reliable methods for their characterisation and toxicity screening. For this purpose, the understanding of the behaviour and fate of these NMs when in contact with biological systems is important. Physical and chemical properties such as size, surface chemistry and surface charge have been identified as essential parameters to determine, because they affect the NMs' mode of action in a given environment (water, buffer, biological fluid, etc.) through different surface molecular interactions.

In particular, hydrophobicity is considered as an important property since it has a critical role in various biological processes such as protein adsorption,² interaction with biological membranes,³ cellular uptake,^{4,5} immune response,⁶ and haemolytic effect.⁷ It is recognised that hydrophobicity is a key property to be controlled for nanomedicine applications since it has a direct influence on the stability and bio-distribution of nanovectors.^{3,6,8} Only a few methods are currently available for characterising the hydrophobicity of NMs⁹—for example surface adsorption assays,¹⁰ NMs relative affinity for reference phases, and hydrophobic interaction chromatography.¹¹ None of those methods enables, for all NM types, a full characterisation and quantification of the NMs hydrophobicity and they all involve expensive and time-consuming analytical techniques. The development of a fast and reliable general method for NMs hydrophobicity characterisation is therefore of great interest.

We described in a previous publication¹² a new method for the separation of NMs according to their hydrophobicity based on a set of collectors composed of fluorinated hydrophobic surfaces whose surface energy components can be modified and fine-tuned by the layer-by-layer (LbL) deposition of polyelectrolytes. A proof-of-concept study of the method was carried out with hydrophobic and hydrophilic polystyrene nanoparticles. The experimental results were qualitatively supported by the eXtended Derjaguin Landau Verwey Overbeek (XDLVO) theory,¹³ enabling the assessment of the different forces in play.

Here we extend this method to quantitatively determine the surface energy components of the NMs by measuring their binding affinity to the collectors' surfaces, via analysis of their adsorption kinetics. The adsorption kinetics is calculated by measuring the number of nanoparticles binding to the different collector as a function of time, by Dark-Field microscopy. The method is particularly suitable for nanoparticles with large light scattering capability (for example noble metals with typical size >50 nm), but it is also applicable to other materials such as SiO₂ with typical size >200 nm. On the other hand, by using a special dark-field condenser¹⁴ or other single-particle microscopy techniques¹⁵ it is in principle possible to detect NMs of any material and down to sizes of the order of 20 nm.

The surface energy potential acting between each NM and the collector is then calculated using the XDLVO theory. The energy barrier potential between the NMs and the collector represents the potential energy at which the NMs are repelled by the collector surface.¹⁶ This energy barrier potential is inversely proportional to the binding affinity: the larger the energy barrier potential, the lower the affinity. In some conditions, the NMs may be completely repelled by the collector due to very high energy barrier potential. Electrostatic forces are mainly responsible for the formation of the energy barrier; hence for an accurate calculation, the Z-potential for both the surfaces and the NMs should be rigorously measured.

The NM's surface energy and in particular the degree of hydrophobicity is then calculated by comparing the NM binding affinity of two or more collectors. The experiments were

performed with Au and SiO₂ nanoparticles (NPs) with different degrees of hydrophobicity to test the validity of the approach. An advantage of this method is that it can cover all the surface energy range with only one set of collectors, and a quantitative determination of the surface energy is possible thanks to several measurements on different collectors.

Results

Surface characterisation of collectors. The first step of the method is to determine the binding affinity of the AuNPs with the different collector surfaces. Each collector prepared as described above is characterised by distinct surface energy components that control the binding affinity of the nanomaterial. First, the binding kinetics of AuNPs was measured on each collector, in a buffer solution (Phosphate Buffer, PB 10 mM, pH = 7). The buffer composition has been chosen in order to partially neutralize the charges present on both the surface and the AuNPs, which would otherwise lead to a long-range repulsion. The principle of the method is described in Fig. 1.

The selectivity and specificity of the AuNPs binding to the surfaces strongly depend on characteristic of the interaction forces such as interaction strength as a function of distance and attractiveness and repulsiveness.

The collector and nanoparticles characterisation, the study of the binding affinity of the AuNPs on the different collectors, calculation of the acting potential between the AuNPs and the collector surfaces, and extraction of the surface energy component of the AuNPs from the adsorption rates of the AuNPs on the collectors surfaces are presented below.

In order to fabricate the collectors with different surface properties, a standard Si wafer has been coated first with a plasma deposited layer of polytetrafluoroethylene PTFE (hydrophobic) and then with several layers of polyelectrolytes (PE) (poly (diallyldimethylammonium chloride, PDPA and polysodium 4-styrene sulphonate, PSS) for giving the surface a more hydrophilic character. The subsequent adhesion of PEL layer also permits the modification of the surface free energy components of the collectors. Five surfaces denominated T0 (PTFE), T1, T2, T3 and T4 are thus produced with the numerical component of the name corresponding to the number of PEL layers deposited.

According to the Owens–Fowkes–Wendt (OFW) theory,^{17,18} the total surface energy of a solid is the sum of the dispersive component (taking into account the non-polar interactions), called γ^{LW} (Lifschitz–van der Waals component) and of a polar component γ^{AB} (acid–base component). Solid materials with low γ^{AB} are called “hydrophobic”. The increase of the γ^{AB} of a solid corresponds to an increase of its hydrophilicity. The PTFE was chosen because of its very low γ^{AB} . The surface free energy components have been determined by measuring the contact angle of the solid surfaces with a polar (water) and a dispersive solution (Bromonaphthalene).

The surface energy values of the as-deposited PTFE were 0.9 mJ/m² and 19.3 mJ/m² for respectively polar and dispersive components. Then, PEL layers were deposited on the collector to increase both surface energy components. The deposition of the cationic PEL leads to a decrease of the initial PTFE z-potential (−60 mV) to values close to zero, while the anionic PEL deposition does not change the negative z-potential (−60 mV). Surface properties of the PTFE and of the T2 collectors present very similar z-potential values while the T2 collector has a higher surface free energy component value, i.e. polar component increasing by a factor 5 and the dispersive components increasing by only 1.4.^{12,19} The increase of the surface free energy has been directly measured by the F–D curves using different functionalized gold-coated tips (Supplementary Figure 1).

The adhesion force between the PFT (polyfluorotetraethylene) modified Au-coated silicon tip and respectively the PTFE and T2 collector were measured as 642 ± 200 nN and 248 ± 100 nN (Supplementary Figure 2). This higher adhesion force between the PFT-modified Gold on the tip and the hydrophobic PTFE is attributed to the hydrophobic interacting forces resulting from their very low polar surface energy component (the tip-surface new interface is energetically more favourable than the two surfaces alone). The average adhesion force of a polyethylene oxide (PEO)-modified Au-coated tip on both the PTFE and the T2 collector (not shown) was 100 ± 76 nN indicating that the hydrophilic surface functionalization reduces the strength of interaction between the surfaces, consequently reducing their force of adhesion.

The atomic force microscopy (AFM) analysis indicates that collector surfaces are rather homogenous with roughness increasing from 0.29 nm to 0.85 nm showing that the morphology of the surfaces is not dramatically affected by the formation of the polyelectrolytes layers. The z-potential was measured for different pH. A negative z-potential was obtained for the whole range of pH, especially for the PTFE non-modified and the PSS layers, and closer to neutral for the different PDPA layers. This result can be

explained by the fact that the PDPA is positively charged and the PSS and PTFE negatively charged.

A more comprehensive overview of the surface characterisation of the collectors is given in ref.¹².

Finally, the set of collectors presents the following features:

1. Relatively low surface roughness, root mean square (RMS) roughness <2 nm.
2. Surface chemical homogeneity, as indicated by the X-ray photoelectron spectroscopy (XPS) and time-of-flight secondary ion mass spectrometry (ToF-SIMS) analysis.¹²
3. Surface homogeneity at the nanoscale level in terms of the adhesion properties as indicated by the force spectroscopy measurements.
4. A surface Zeta-potential which is negative (for the PSS terminated surfaces) or slightly negative (for the PDPA terminated surfaces).
5. Different values of LW and AB components.

Nanoparticles characterisation. Supplementary Figure 3 illustrates typical examples of transmission electron microscopy

Fig. 1 The proposed method for quantification of the hydrophobicity of nanomaterials. **a** To form a colloidal suspension, hydrophobic NMs are stabilised by hydrophilic ligands to prevent the aggregation of the material due to hydrophobic attractive forces. After incubation with collectors, characterised by the same surface charge but different hydrophobicity, the XDLVO energy drives the stable adsorption of the nanomaterial to the collector 1, while the particles are desorbed in case of collector 2. **b** The different positions and strengths of the energy barrier are highlighted. **c** Simulated rate of adsorption curves for the two collectors with respect to the maximum adsorption rate V_{max} occurring in the absence of the energy barrier

Table 1 Determination of the PEO surface coverage of the AuNPs

AuNP sample	A	B	C	D	E	F	G	H	I	L
Volume of added PEO (μl)	0	0.16	0.32	0.48	0.72	1.04	1.6	3.2	4.8	7.2
UV-vis peak spectral position (nm)	543.3	546.2	546.4	546.4	-	546.6	546.7	-	-	546.9
DLS hydrodynamic diameter (nm)	62.7 $\pm$ 10	-	-	63.1 $\pm$ 10	-	63.5 $\pm$ 10	64.9 $\pm$ 12	-	-	68.7 $\pm$ 15
Z-potential (mV)	-45 $\pm$ 2	-42 $\pm$ 2	-40 $\pm$ 2	-41 $\pm$ 2	-	-40 $\pm$ 2	-36 $\pm$ 2	-	-	-39 $\pm$ 2
DCS peak of the runtime (s)	9.71	9.94	9.91	-	9.99	10.08	10.18	10.41	10.51	10.62
Number of PEO molecules per AuNP (1E4)	0	5	16	20	18	-	-	55	194	210
W/O partition, % of NPs in water	0	25	38	36	68	77	92	-	-	100
SDS-PAGE, intensity of 40–200 KDa band (relative to pure HSA) (%)	7.17	-	-	-	4.77	1.34	-	0.05	0.02	0
PEO surface coverage (theoretical) (%)	0	5	10	15	22.5	32.5	50	100	$\geq$ 100	$\geq$ 100

Summary of the characterisation of the AuNPs functionalized with self-assembled PEO at different theoretical surface coverage

(TEM) images obtained by spotting 4 μl AuNPs stock solution onto a C/Formvar TEM grid and dried overnight in air. Although the morphologies observed were mainly spherical, a certain degree of faceting characteristic of large AuNPs was also observed. Size distributions determined by TEM image analysis on over than 100 objects were narrow with an average value of 60.2 ± 7.9 nm.

The AuNPs were functionalized with different amounts of PEO-ligands in order to produce different surface coverage on the surface i.e. different hydrophobic characters. The selected ligand chain was functionalised with a SH-group at one terminus to provide a strong affinity for gold, a central non-polar alkyl chain to provide to the structure the ability to self-assemble in dense layer that excludes water due to the hydrophobic core and a PEO sequence to enhance stability in water.

The PEO surface coverage has been first characterised by UV-vis adsorption measurements (Supplementary Figure 4). The results show that the increase of PEO content in the solution results in a red-shift of the surface plasmon resonance wavelength from 543 nm for uncoated to approximately 546 nm for all the functionalized PEO-Au NP samples (Table 1). The shift from the uncoated and the PEO coated surfaces might be due to slight differences in the bulk refractive index of the dispersant.

Dynamic light scattering (DLS) also confirms the successful functionalization of AuNPs 60 nm with an increase of the hydrodynamic diameter from 62.7 nm for pristine AuNPs to 68.7 nm for the highest PEO concentration (Table 1). Z-potential measurements show a slight decrease in the negative surface charge from -45.7 mV for pristine AuNPs to -39.4 mV for the AuNPs coated at the highest PEO concentration. Differential centrifugal sedimentation (DCS) shows that the sediment time increases with the PEO concentration in the solution. This shift toward higher sedimentation times is the net result of (1) the increase of NP diameters and (2) the decrease of the NP apparent densities due the PEO binding. By combining the sedimentation times measured by DCS and the DLS diameter,²⁰ the mass of absorbed PEO molecules on the AuNPs as a function of the PEO concentration in solution and therefore the number of molecules per NP can be calculated. The values are reported in Table 1.

A theoretical full coverage of the AuNPs by the PEO molecules can be calculated by dividing the available gold surface area of the particles by the footprint of an alkanethiolate molecule ($21.4 \text{ \AA}^2$), i.e. the minimum space occupied by an absorbed molecule as estimated by electron diffraction studies of monolayers of alkanethiolates on Au(111) surfaces.²¹

We must underline that the obtained value of the coverage represents a rough approximation, since it corresponds to a perfect packing of the ligand on the surface and doesn't take into account physical effects such as the steric repulsion of the PEO chains, the gold surface inhomogeneity, the possibility different

orientation due to the PEO chains interaction with the gold surface, occupying more space, etc. Using the TEM diameter (60.2 nm) and considering the AuNPs as perfect spheres, we calculated a maximum number of 55×10^4 PEO molecules per AuNP. According to Table 1 this number is reached for a volume of PEO added to the AuNPs of 3.2 μl . Any additional PEO added to the solution would potentially lead to the formation of an excess of PEO on the surface of the AuNPs, i.e. multilayers.

More interestingly, the PEO functionalized AuNPs were characterised by two other techniques: protein adsorption by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and affinity to the water phase by water/octanol partition.

The amount of proteins detected by the SDS-PAGE on the AuNPs was very low, since the total amount of AuNPs available was very small. However, it was found that a detectable quantity of Albumin was found on both the pristine AuNPs and the AuNPs treated with low volumes of PEO (Supplementary Figure 5, Supplementary Note 2). By plotting the SDS-PAGE Albumin band intensity as a function of the volume of added PEO, it is clear that the intensity decreases and it goes to zero for a PEO volume larger than 3 μl . This means that the PEO surface coverage obtained by adding a volume of 3 μl to the solution was sufficient to prevent any protein adsorption on the surface of the particles.

A similar trend is observed in the water/octanol partition coefficient. Briefly, experiments were carried out both with pristine AuNPs of 60 nm and the differently PEO-functionalized AuNPs at a concentration of 0.5 mM of gold. As expected, pristine hydrophobic AuNPs coated with weakly bound citrate surfactant interacted strongly with octanol resulting into complete aggregation clearly visible in the interphase (Supplementary Figure 6). By increasing the degrees of PEO-functionalization, a progressive decrease in the amount of aggregation together with higher amount of NPs in the aqueous solution can be observed (increases of red colour intensity). For volume of added PEO larger than 3 μl , the AuNPs did not interact at all with Octanol as a result of their hydrophilic character. These results confirm the decrease of hydrophobicity as the PEO coverage increases and a saturation of the surface for 3 μl of thiolate added to the suspension. The proportion of NPs in water was calculated by measuring the intensity of the red colour component in Supplementary Figure 6 for each vial and normalizing to the intensity of the red colour of the original solution of NP with the same concentration in water.

These two results suggest that, as calculated by DCS/DLS combined measurements, a full monolayer of PEO is reached by adding a volume of PEO larger than about 3 μl to the pristine AuNPs solution. The addition of larger volumes of PEO did not further modify the surface properties of the AuNPs. The results from these three experiments are summarized in Fig. 2.

Fig. 2 Effect of increasing PEO coating on nanoparticles behaviour. Summary of the PEO functionalized AuNPs characterisation as a function of the PEO volume added to the pristine AuNPs solution. **a** Square symbols represent the estimated PEO surface coverage of the AuNPs, and the indication of the saturation reached at a volume of added PEO equals to 3.2 μl . **b** In inverted triangles, the results from SDS-PAGE are shown, indicating that the signal related to the HSA binding drops to zero for volume of added PEO equals to 3.2 μl . **c** In triangles, the relative concentration of AuNPs in water in water/octanol experiment is presented

Furthermore, we characterised the hydrophobicity/hydrophilicity degree of the Au-Citrate stabilised and of the Au-PEO NPs, by contact angle measurements performed on a dried film of NPs.

Contact angle measurements show that Au-citrate NPs are hydrophobic with a corresponding contact angle with water of about 95° . The AuNPs with the addition of 7.2 μl of PEO are on the other hand very hydrophilic, with a contact angle of 23° . A similar behaviour was observed on the flat Au surface, where the pristine Au surface showed a contact angle of 84° with water and the PEO-functionalized Au flat surface a contact angle of 40° .

The previously described combination of characterisation techniques of the AuNPs allowed us to classify the AuNPs according to their effective PEO surface coverage. The surface coverage is linearly proportional to the amount of PEO added to the pristine AuNPs solution between 0 and 3.2 μl of volumes. The proportionality factor, k , between volumes was added and the PEO surface coverage was calculated as follows:

$$k = \frac{100\%}{3.2} = 31.25\%/\mu\text{l} \quad (1)$$

The AuNPs samples A, B, C, D, F, G and L, corresponding to the PEO surface coverage indicated in Table 1 were used to study the binding affinity with the different collectors.

Nanoparticles binding study. The AuNPs affinity has been calculated by directly measuring the real-time binding kinetics of the NPs to the different collectors. The binding curves are built by using a script created in the software ImageJ allowing to automatically detecting the NPs as round objects in the video sequence. Typical sequence images of the adsorption of NPs on the T0 collectors are shown in Fig. 3, for the Au-citrate NPs (hydrophobic) and for the Au-100% PEO coated NPs (hydrophilic). Each round object in each frame corresponds to one AuNP; for each frame, the NPs are counted and the position of each NP is recorded. It is either possible to detect the total number of NPs for each frame or to track the NP motion on the

surface. Only the NPs perfectly in focus with the surface plane are detected with a focal depth of 4000 nm. Taking this into account the nanoparticles were detected in the volume above 4000 nm from the surface: these particles are moving much faster than the time resolution of the camera ($> \text{ms}$). Hence we are able to observe only the nanoparticles slowed down by the contact with the surface, which are moving or rolling. The number of NPs detected is then related to the affinity of the NPs to that particular surface.

It should be noted that AuNPs with different PEO coverage are characterised by the same core size and a similar negative Z-potential. The choice of negatively charged collectors (terminated by the anionic polyelectrolyte) and buffer conditions was made in order to minimize any possible electrostatic interaction between the nanoparticles and the surfaces while keeping the stability of the colloids. Uncoated Au-citrate NPs dispersion stability is maintained also thanks to their very negative value of the z-potential while the increasing PEO coating coverage contributes to the NPs stability by steric repulsion. In the absence of an energy barrier, the number of NPs reaching the surface as a function of time $\Gamma^{\text{Ads}}(t)$ is determined by the diffusion constant, D_e via Eq. 2:

$$\Gamma^{\text{Ads}}(t) = 2C_n \Sigma \left(\frac{tD_e}{\pi} \right)^{1/2} \quad (2)$$

Where Σ is the area of measurement and C_n is the bulk concentration of NPs. The maximum velocity of adsorption v_{max} of the NPs of radius r and bulk concentration C_n on an area Σ is then

$$v_{\text{max}} = \frac{\Gamma^{\text{Ads}}(t)}{\sqrt{t}} = 2C_n \Sigma \left(\frac{kT}{6\eta\pi^2} \right)^{1/2} \left(\frac{1}{r} \right)^{1/2} \quad (3)$$

Eq. 3 shows that, in the absence of an energy barrier, the NPs adsorption velocity is determined by the radius of the NPs, through their diffusion constant.²² If an energy barrier, ΔG^{max} , is present, the local concentration of NPs, C_{loc} at a distance lower than the energy barrier distance is related to the bulk concentration by Eq. 4:

$$C_{\text{loc}} = C_n e^{-\Delta G^{\text{max}}/kT} \quad (4)$$

Eq. 4 is the classical Maxwell-Boltzmann distribution which determines the distribution of objects in the presence of an energy barrier.²²

The generalized NPs adsorption velocity is then

$$v = v_{\text{max}} e^{-\Delta G^{\text{max}}/kT} \quad (5)$$

That substantially means that the NP adsorption velocity is reduced by a factor $e^{-\Delta G^{\text{max}}/kT}$ in the presence of an energy barrier as compared to the maximum velocity.

The v_{max} can be calculated by Eq. 3, knowing the size of the NPs or it can be measured directly by observing the rate of arrival of the negatively-charged NPs to a positively-charged surface (such as T1 or preferably T3 since one layer of polyelectrolyte on the PTFE is not sufficient to guarantee full surface coverage).

On the other hand, by measuring the velocity in the presence of an energy barrier one can calculate $\Delta G^{\text{max}}/kT$ as

$$\frac{\Delta G^{\text{max}}}{kT} = -\ln \frac{v}{v_{\text{max}}} \quad (6)$$

The ratio $\frac{v}{v_{\text{max}}}$ can vary between 0 and 1 and it is a direct

Fig. 3 Measuring binding kinetics. Automatic detection of the AuNPs absorbed on the T0 (hydrophobic) collector by means of the macro developed with ImageJ software as a function of time after the injection of the nanoparticles. **a** Show the time evolution for the citrate stabilised AuNPs **b** and for the AuNPs with 100% of PEO coating

measurement of the energy barrier present between the NPs and the collector.

For the 60 nm AuNPs the maximum adsorption rate is obtained when the activation energy barrier is zero, and the exponential in Eq. 5 is equal to 1.

In the cases where the gravitational transport is not negligible for the timescale observed (dense and large NPs) Eq. 2 should be corrected with a factor proportional to the time and the sedimentation velocity.

$$\Gamma^{\text{Ads}} = 2C_n \Sigma \left(v_{\text{sed}} t + \left(\frac{t D_e}{\pi} \right)^{1/2} \right) e^{-\frac{\Delta G^{\text{max}}}{kT}} \quad (7)$$

$$\text{where } v_{\text{sed}} = \frac{2g(\rho_{\text{NP}} - \rho_f)r^2}{9\eta} \quad (8)$$

with ρ_{NP} and ρ_f being the NP and fluid mass density respectively, and η the fluid viscosity. Eq. 7 takes into account that the local concentration of the NPs is increasing with time due to the sedimentation of the NPs.

The adsorption curves are fitted by a modified Eq. 9:

$$\Gamma^{\text{Ads}} = \alpha_1 t^{1/2} + \alpha_2 t \quad (9)$$

where α_1 and α_2 are respectively the linear and quadratic factors (for the polynomial function in $t^{1/2}$).

The maximum adsorption rate which depends on the geometrical properties of the NPs is plotted in Fig. 4 in comparison with the kinetics obtained for the different NPs for the different collectors. The rate of adsorption of the NPs on the different collectors is decreasing while increasing the acid-base (polar) component of the collectors. This is more evident for the non-PEO-coated NPs. Then the rate of adsorption is found decreasing drastically with the increasing PEO% coating on the NPs. For a nominal coating of PEO larger than 25% of the NPs, the adsorption rate is close to zero.

The principal information that can be extracted from Table 2: on the T4 collector, the rate of adsorption is zero for all the NPs,

Fig. 4 Measured kinetics curves. Association curves for the hydrophobic NPs on the three collectors as compared to the maximum adsorption rate calculated from the theory. The red lines represent the best-fit of the association curves using the function in Eq. 9. The fitted values for the linear part (α_1) are presented in Table 2

Table 2 Measuring nanoparticles binding kinetics. Values for α_1 on the different collectors fitted from the association curves using Eq. 9

Sample	α_1 T0 (PTFE) (NP/s ^{1/2})	α_1 T2	α_1 T4
Au-cit	1.85 ± 0.18	0.65 ± 0.17	0.05 ± 0.05
Au-PEO 5%	1.40 ± 0.15	0.38 ± 0.09	0.05 ± 0.05
Au-PEO 10%	1.18 ± 0.18	0.32 ± 0.18	0.05 ± 0.05
Au-PEO 15%	0.77 ± 0.18	0.27 ± 0.10	0.05 ± 0.05
Au-PEO 32.5%	0.12 ± 0.08	0.09 ± 0.06	0.05 ± 0.05
Au-PEO 50%	0.05 ± 0.05	0.05 ± 0.05	0.05 ± 0.05
Au-PEO 100%	0.05 ± 0.05	0.05 ± 0.05	0.05 ± 0.05

the rate of adsorption is larger on the collector T0 than on the collector T2 for all the NPs and the difference of the rate of adsorption between T0 and the collector T2 is decreasing by increasing the PEO coating.

Calculation of the surface free energy component of the nanoparticles. According to the XDLVO theory²³, the total interaction energy G^{tot} can be expressed as

$$G^{\text{tot}} = G^{\text{el}} + G^{\text{AB}} + G^{\text{LW}} \quad (10)$$

where G^{el} , G^{AB} and G^{LW} are energies relative to the electrostatic, acid-base and Lifshitz–Van der Waals interactions respectively. The three potential depends on the distance between the NP and the surface.

Electrostatic interaction energy is

$$G^{\text{el}} = \pi \epsilon R_N (\zeta_N^2 + \zeta_S^2) \left[\frac{2\zeta_N \zeta_S}{\zeta_N^2 + \zeta_S^2} \text{Ln} \frac{1 + \exp(-\kappa d)}{1 - \exp(-\kappa d)} + \text{Ln}\{1 - \exp(-2\kappa d)\} \right] \quad (11)$$

where d is the separation distance between the NP and the surface, ζ_N and ζ_S are the surface charge of the nanoparticle and the collector surface respectively. $1/\kappa$ is the double layer thickness, which is expressed from Eq. 12.

$$\kappa = \left[\frac{e^2}{\epsilon k T} \sum_i z_i n_i \right]^{1/2} \quad (12)$$

where ϵ is the permittivity of the medium, e is the charge of electron, k is the Boltzmann constant, T is the temperature, z_i is the valency of the ions i , and n_i is their number per unit volume.

The Lifshitz–Van der Waals ΔG^{LW} components to the free energy of interaction between a nanoparticle and surface are calculated following the extended DLVO theory:

$$G^{\text{LW}} = -\frac{H}{6} \left[\frac{2r(d+r)}{d(d+2r)} - \text{Ln} \frac{d+2r}{d} \right] \quad (13)$$

where d is a separation distance between the NP and the surface, and r is the radius of the nanoparticle.

H the effective Hamaker constant for the NP–collector–water system, which can be expressed as

$$H = 24\pi d_0^2 \left(\sqrt{\gamma_N^{\text{LW}}} - \sqrt{\gamma_w^{\text{LW}}} \right) \left(\sqrt{\gamma_s^{\text{LW}}} - \sqrt{\gamma_w^{\text{LW}}} \right) \quad (14)$$

Looking at the dependence of the G^{LW} on the distance d , the Lifshitz–Van der Waals force then active for very short distances ($d < 1$ nm), only for the particles that are able to overcome the energy barrier.

While the analytical expressions for the electrostatic potential and the Lifshitz–Van der Waals potentials are well known and commonly accepted, the acid–base interaction potential is mainly represented as an empirical formulation based on experimental observations²⁴ and direct measurements of the interaction potential between two surfaces (sphere–sphere, sphere–plane, plane–plane) in a polar medium or in an electrolytic solution. The G^{AB} is including all forces involving the structural reorganization of the water molecules around two surfaces, depending on the degree of wettability of the surfaces involved. For a sphere–plane system

$$G^{\text{AB}} = \pi r \lambda \Delta G^{\text{AB}} e^{[(d_0 - d)/\lambda]} \quad (15)$$

where d_0 is the minimum separation distance between the NP and the surface, taken generally as 0.158 nm for many different

kinds of substrates and d the separation distance in nm. G^{AB} is defined as a short-range acting potential, having an exponential decrease with the distance. The field of interaction of the potential is mainly determined by the correlation length λ , expressed in nm. Various values for λ have been reported in literature, ranging from 0.2 nm to up to 13 nm²⁴.

The nature of the two interacting surfaces intervenes in the AB potential with the term ΔG^{AB} , which can be expressed as

$$\Delta G^{\text{AB}} = -2 \left(\sqrt{\gamma_N^{\text{AB}}} - \sqrt{\gamma_w^{\text{AB}}} \right) \cdot \left(\sqrt{\gamma_s^{\text{AB}}} - \sqrt{\gamma_w^{\text{AB}}} \right) \quad (16)$$

where the term $\sqrt{\gamma_i^{\text{AB}}}$ refers to the polar component of the surface free energy for N = nanoparticle, W = water and S = surface.

The interaction energy maps were calculated using the function wizard included in the software OriginPro 2015. The values for the interaction energy are given in kT units ($1 \text{ kT} = 4 \times 10^{-21} \text{ J}$). The total interaction energy is expressed in Eq. 10 for the interaction of a NP and a surface as a function of the distance and of the hydrophobic correlation length λ . Even if the range of possible and measured value in literature for λ is broad (between 0.6 nm and 13 nm), we decided to keep it in the range between 0.6 nm and 2 nm, in order to take into account possible influences of the radius of curvature of the NPs and of the roughness of the collectors²⁴. The heat-maps for the 15 nm, 60 nm and 200 nm hydrophobic NPs on hydrophobic collector are shown in Supplementary Figure 7 and illustrate the influence of the value of λ on the potential profile as a function of the NP size (Supplementary Note 3). The blue colour map indicates that the interaction energy between the NPs and the surface is equal to or lower than 0 kT. When interaction energy between the NP and the surface is equal to or larger than 5 kT the colour map is marked as red. 5 kT is considered as the threshold value for an energy barrier that would inhibit completely the adsorption of NPs to the surface. The corresponding adsorption velocity would be $v_{5 \text{ kT}} = 0.0067 \cdot v_{\text{max}}$.

The heat maps for 60 nm bare AuNPs with T0 hydrophobic and T2 and T4 hydrophilic collectors are presented in Fig. 5.

To illustrate the variation of the energy barrier, in assuming a value of 1.65 nm for λ , (dashed line in Fig. 5) the energy barrier maximum is found lower for the T0 (about 0.5 kT) than for the T2 and T4 collectors. The position of the maximum in z is also depending on the collector, being larger for T0 (about 13 nm) and smaller (or closer to the surface) for T2 and T4.

For each collector, the energy barrier γ_N^{AB} must verify the following system of non-linear equations:

$$\begin{cases} G^{\text{tot}, T_i}(z_i, \lambda, \gamma_N^{\text{AB}}) = \frac{\Delta G^{\text{max}}}{kT} = -\ln \frac{v_i}{v_{\text{max}}} \\ \frac{\partial G^{\text{tot}, T_i}(z_i, \lambda, \gamma_N^{\text{AB}})}{\partial z_i} = 0 \end{cases} \quad (17)$$

where the first equation in bracket corresponds to the value of the maximum of the energy barrier and the second equation corresponds to the condition of the maximum (first derivative equals to zero). According to Eq. 6, the maximum of energy barrier determines the reduction of the velocity of adsorption with respect to the maximum velocity (when the energy barrier is zero).

The varying parameters to verify the system in Eq. 17 are: the NPs–surface distance z_i which depends on the collector. The z_i is the NP–surface distance at which the energy barrier has a maximum, as a function of the collector, the hydrophobic interaction characteristic distance, λ and the polar component of the surface free energy of the NPs, γ_N^{AB} .

Fig. 5 Collector-dependent energy barrier. Interaction energy map for hydrophobic bare AuNPs and the three used collectors: **a** for T0, **b** for T2 and **c** for T4. The colour map is plotted as a function of the NP-collector distance and the correlation length, λ . The red area represents a combination of parameters at which the adsorption of particles is forbidden the energy barrier being too large (>5 kT). At $\lambda = 1.65$ nm, the hydrophobic collector, T0, shows the smallest energy barrier compared to T2 and T4

In order to solve the non-linear system of equations in Eq. 17 we need at least two collectors, in order to have four equations with four unknowns, namely z_1 , z_2 , λ and γ_N^{AB} .

The system of equations, for each couple of collectors has been numerically solved using the value boundaries listed in Supplementary Table 1. The boundaries were chosen as follows: z_i is the typical range of interaction of the electrostatic forces; the γ_N^{AB} values were chosen between the value corresponding to PTFE (hydrophobic) and the one of water (the maximum hydrophilicity). The tolerance for the G^{tot} was set at 10% of the

Table 3 Measurement of hydrophobicity. Parameters solving the system of equation in Eq. 17

Sample	Z_{T0} (nm)	Z_{T2} (nm)	λ (nm)	γ_N^{AB} mJ/m ²
Au-cit	12.84 ± 0.59	9.50 ± 0.09	1.69 ± 0.01	1.57 ± 0.45
Au-PEO 5%	12.49 ± 0.18	8.90 ± 0.12	1.69 ± 0.01	7.35 ± 0.76
Au-PEO 10%	12.16 ± 0.11	8.56 ± 0.06	1.69 ± 0.01	9.47 ± 1.20
Au-PEO 15%	11.26 ± 0.13	7.66 ± 0.04	1.69 ± 0.01	13.20 ± 2.10
Au-PEO 32.5%	9.69 ± 0.05	6.04 ± 0.04	1.68 ± 0.01	21.84 ± 2.74
Au-PEO 50%	9.42 ± 0.40	5.76 ± 0.32	1.73 ± 0.03	30.95 ± 4.11
Au-PEO 100%	9.42 ± 0.40	5.76 ± 0.32	1.73 ± 0.03	30.95 ± 4.11

Fig. 6 Direct measurement of the hydrophobicity as a function of the PEO coverage. Correlation between the PEO surface coverage of the AuNPs and of the polar component of the surface energy of the NPs (γ_N^{AB}) calculated with the present method. The error bars in the plot were calculated by spreading the errors for the determination of the binding velocity of the particles on the different collectors

value calculated between a hydrophilic particle and a hydrophilic collector.

The numerical solution for the T0–T2 couple of collectors for the different AuNPs is listed in Table 3.

The results obtained from the solution of the system of equations for the T0–T2 couple of collectors are physically meaningful. The fitted value for λ is very similar for the different NPs and it is very close to the one obtained graphically from Fig. 5. A value for each NP of γ_N^{AB} is then determined with this method. According to what was expected and also measured by the contact angle on the dried particles pellets, the γ_N^{AB} is the lowest for the Au-citrate NPs (1.57 mJ/m²) and increases for the PEO coverage percentage. For coverage larger than 25% the AuNPs are largely hydrophilic with a large value for the polar component of the surface free energy (30.95 mJ/m²). The graphical evolution of the γ_N^{AB} with the PEO coverage is shown in Fig. 6.

A second type of model NPs has been used, to verify the validity of the proposed method. 200 nm hydrophilic SiO₂ NPs were modified with different chemical groups to increase the surface hydrophobicity. Three functional groups were used to modify the NP surface, consisting of alkyl chain of different length: propyl, butyl and dodecyl groups. The SiO₂ NPs modified with dodecyl groups were not stable and started to aggregate, forming dimers and trimers as shown by the DLS measurement.

Table 4 Behaviour of the modified silica particles. Parameters solving the system of equation in Eq. 17 for the T0–T2 collectors with the hydrophilic silica NPs

Sample	Z _{T0} (nm)	Z _{T2} (nm)	λ (nm)	γ _N ^{AB} mJ/m ²
SiO ₂ -propyl	10.42 ± 0.45	6.15 ± 0.32	1.81 ± 0.03	30.47 ± 4.15
SiO ₂ -butyl	10.32 ± 0.52	6.10 ± 0.21	1.81 ± 0.03	30.81 ± 5.56

The propyl and butyl functionalized SiO₂ NPs were stable for days as shown by DLS measurement.

The adsorption rate of pristine SiO₂ and epoxy modified NPs on T0 (and consequently on T2 and T4) was zero.

The adhesion of propyl and butyl functionalized SiO₂ NPs to T0, the hydrophobic collector, was slow but different from zero, and it was evaluated as $\alpha_1 = 2.15 \text{ t}^{-1/2}$ and $\alpha_1 = 2.17 \text{ NP/t}^{-1/2}$, respectively. The maximum adsorption rate, calculated with Eq. 2, is $232 \text{ NP/t}^{1/2}$, meaning that the potential barrier is relatively large, around 6.97 kT and 6.98 kT. The results are summarized in Table 4.

This result is confirmed by the contact angle measurement with water of a silica surface functionalized with propyl and butyl groups: the contact angle is about 10° and 15° for the propyl and butyl, respectively, confirming the highly hydrophilic nature of these surfaces.

Discussion

In this work a method is presented for the quantitative characterisation of nanoparticles hydrophobicity by measuring their affinity towards specifically functionalized surfaces.

The determination of the affinity of NPs towards substrate surfaces with different hydrophobicity degrees enables the direct characterisation of the NPs having unknown surface functionalization and residual hydrophobicity in a direct manner. The quantification of the surface energy of the NPs is possible by comparing the evaluation of the affinity of the NPs with different collectors and the comparison with the XDLVO theory, which takes into account the hydrophobic forces.

The general method is highly sensitive to variation of the surface free energy polar components and could be adapted for the direct evaluation of the stability of the anti-fouling coatings which are usually used to prevent the agglomeration of the NPs in biological complex media and living bodies and which, through the formation of the protein corona, may lead to inflammatory responses and/or uncontrolled clearance of the NPs. The proposed method allows the quantitative characterisation of NP hydrophobicity in solution and thus is potentially highly relevant to important applications in the field of nanomaterial safety assessment in consumer and industrial products.

Methods

Surface preparation. Silicon wafers (Si(100); diameter, 50 mm; resistivity, 1–20 Ω cm) supplied by ITME (Warsaw, Poland) were used as the substrate for the whole study. Before modification the wafers were washed with ethanol and water and dried under nitrogen flow.

Surface modification. The silicon substrate was modified by different deposition processes in order to tune the surface hydrophobicity. First, polytetrafluoroethylene (PTFE) coating was plasma deposited to generate a hydrophobic surface. The deposition was performed using pure octafluorocyclobutane (C₄F₈) as the gas precursor at a pressure of 3.5 Pa, applying a power of 142 W for 5 min¹².

In order to tune the surface hydrophobicity, a layer-by-layer deposition of two polyelectrolytes (PELs) was then performed. The PTFE modified substrates were incubated for 2 min in poly(diallyldimethylammonium chloride) (PDDA) 2% solution in water or in poly(sodium 4-styrene sulphonate) (PSS) 2% in water for the self-assembly deposition of each PEL layer, starting from PDDA (positively charged) and alternating with PSS (negatively charged). After each step, the substrate was rinsed with milliQ water and dried under nitrogen flow. The surfaces

obtained are referred to as T1, T2, T3 and T4 depending on the number of PEL layers deposited.

Collector surface characterisation. In order to fully characterise the collector surfaces, several techniques were used. The thickness and refractive index of each deposited layer were measured by Ellipsometry (Vase VUVTM J.A. Woollam Co.). The contact angle and surface energy of the surfaces were determined by using a DigidropTM goniometer with 2 liquids (water and 1-bromonaphthalene). The surface topography and roughness of the surfaces were measured using AFM (NT MDT Russia). Finally, Z-potential measurements were performed for a range of pH from 3 to 10 with a step of 0.5 in order to characterise the surface charge. The Z-potential was measured for independent samples just after adjusting the pH of the dispersions with either 1 M NaOH or 1 M HCl and at a total NaCl concentration of 1 mM to keep the conductivity at approximately 1 mS/cm.

AFM was also used to directly measure the different adhesion forces present between the collector surfaces and functionalised tips to ensure that the surface properties evaluated were constant over a 1 μm range. A conventional silicon tip for contact mode (NT MDT C-Probe, spring constant $k = 0.02 \text{ N/m}$ and nominal radius of curvature of 15 nm) was employed. The instrument returns the values for the deflection of the cantilever in nA (the difference of current at the 4-quadrant optical detector of the AFM). This value was translated in force values (nN) by calculating the actual deflection of the cantilever in nm and by multiplying the value for the elastic constant of the cantilever used.

The cantilever was coated with a 20 nm-thick layer of gold by magnetron sputtering (a 1 nm layer of titanium was previously deposited to ensure the adhesion between the gold and the silicon). The gold-coated tip was then immersed in 1 mM ethanol solutions of thiolated alkyl group terminated with PEO (Polyethylenoxide). The tip was then rinsed in ethanol and ultrapure water and gently dried in flowing N₂. The gold-coated and PEO-functionalised tip was then used to perform so-called “Force–distance curves” (F–D curves). Briefly the tip was brought in contact with the surface in a certain location, and then the tip was retracted by a few micrometers by means of a piezoelectric crystal. Then a series of approaching–retracting curves were acquired in an area surrounding the contact position and the deflection of the cantilever was recorded as a function of the piezo-position. The measurements were performed in ultrapure water. A minimum of 100 curves was acquired. The adhesion force between the tip and the surface was measured for each curve as the difference between the zero-force line and the minimum of the “snap-off” force. The adhesion forces were then averaged for all the curves (see Supplementary Note 1).

Nanoparticle synthesis functionalisation and characterisation. Two sets of NPs, Au and SiO₂ were used for the experiments. AuNPs were synthesised (see below) using gold (III) chloride trihydrate (>99.9%), and trisodium citrate dihydrate (>99.9%). Surface modifications of SiO₂ particles was done using (3-glycidyloxypropyl)trimethoxysilane (GPTMS) (GPTMS, >98%), propylamine (>99%), butylamine (>99%) and dodecylamine (>99.5%). All reagents were purchased from Sigma-Aldrich and used as received without further purification. Ligand 2-(2-[(11-mercapto-undecyloxy)-ethoxy]-ethoxy)-ethoxy-ethoxy-acetic acid (PEO-ligand) was purchased from ProChimia and kept under N₂ and in the freezer at –20 °C. SiO₂ NPs of approximately 200 nm (NS-0200A) at a concentration of $7.8 \times 10^{12} \text{ NP/mL}$ were purchased from MSP Corp. Centrifugal filter units (Amicon, USA) were washed three times with milliQ-water at 3500 rcf 10 min before their use. All solutions were prepared with ultrapure water (Millipore Milli Q system, resistivity, 18.2 Ω cm). The synthesised AuNP dispersions were stored in the fridge at 4 °C.

Synthesis of the AuNPs. AuNPs of 60 nm in diameter were prepared by a seeding-growth four-step procedure. Initially, AuNP seeds of approximately 15 nm in diameter were synthesised with a modified Turkevich method²⁵ using a specialised microwave Discover apparatus (CEM Corporation, USA). Briefly, 5 mL of aqueous gold (III) chloride trihydrate (10 mM) were added to 95 mL of milliQ-water in a single-necked 100 mL round bottom flask equipped with a magnetic stirrer and a glass condenser column. The flask was mounted in the microwave, heated rapidly (<1 min) to 97 °C while stirring and then held for 5 min using a maximum microwave power of 150 W. Under vigorous stirring, 2.5 mL of sodium citrate (100 mM) was injected and the reaction mixture was maintained at 97 °C for 20 min after which the reaction vessel was rapidly cooled to 60 °C with compressed air and then allowed to cool to room temperature. The nominal concentration of gold in the AuNP dispersion was 0.5 mM. The 15 nm AuNP seeds were first grown to 30 nm, and then from 30 nm to 45 nm.

The last stage of the synthesis to 60 nm AuNPs was carried out by the regrowth method from the 45 nm AuNPs. The as-synthesised seeds (25 mL) of AuNPs of 45 nm were diluted in milliQ-water (60 mL) and mixed with 2.8 mL of sodium citrate (100 mM) and 1.25 mL of aqueous gold (III) chloride trihydrate (10 mM). The pH of the resulting solution was adjusted to a value of 9.0 with aqueous NaOH (200 mM, 0.42 mL) and the mixture was heated to 60 °C for 48 h in order to produce AuNPs of approximately 60 nm in diameter. The nominal concentration of gold in final AuNP dispersion was 0.24 mM.

The 60 nm AuNP dispersions were purified twice by centrifugation (2500 rcf, 20 min, 4 °C) followed by redispersion in the same volume of MilliQ water, and

then concentrated by filtration with centrifugal filter units at 100 kDa MWCO (2000 rcf, 2 min, 4 °C). The final approximate concentration was calculated to be 0.5 mM of gold by UV-vis spectroscopy using extinction coefficients of citrate-capped AuNMs determined in the literature²⁶.

Functionalisation of citrate-functionalised AuNPs with different percentages of PEO-ligand. The AuNPs were systematically functionalised with different amounts of PEO-ligands in order to produce different percentages of coating coverage on the surface. Samples of pristine AuNPs of 60 nm in diameter (200 µL, 0.5 mM) were mixed with different volumes (0, 0.16, 0.32, 0.48, 0.72, 1.04, 1.6, 3.2, 4.8 and 7.2 µL) of a solution of PEO-ligand (0.1 mM in milliQ H₂O) to obtain 0, 2.5, 5, 7.5, 10, 15, 25, 50, 75 and 100% theoretical coverage of the total available surface of AuNPs, respectively. The mixtures were stirred at room temperature overnight. The success of the functionalisation was checked by UV-vis spectroscopy, DCS, DLS and Z-potential measurement at a concentration of 0.1 mM of gold.

Functionalisation of SiO₂ NPs with different hydrophobic ligands. Functionalisation of commercial SiO₂ NPs was performed as described in the literature²⁷. Briefly, a sample of nano-silica NS-0200A (0.5 mL) was diluted in water (1.5 mL) and the pH increased with the addition of 1 M NaOH (1 µL). GPTMS (0.5 µL, 2.3 µmol) was immediately added and the reaction mixture was stirred at room temperature for 24 h. Modifications of epoxy-functionalised SiO₂ NPs were obtained by the addition of 100 mM solutions in water of propylamine, butylamine and dodecylamine (final concentrations of 2.24 mM) at pH = 9. The mixtures were left to react for 24 h and all the samples were then purified using centrifugal filter units of 10 kDa MWCO Amicon Ultra-15 (3500 rcf, 5 min) via two washing steps with water in order to eliminate the excess of non-conjugating molecules. DLS measurements showed no major differences in the mean hydrodynamic size for any of the samples, which was a good indicator of the colloidal stability of these solutions. Surface charges of pristine and functionalised SiO₂ NPs were determined by Z-potential measurements at a value of pH = 7.5 after adjusting with 1 M HCl and at a conductivity of 1 mS/cm after adjusting with 1 M NaCl. The final nanoparticle dispersions were diluted in a buffer (Phosphate buffer (PB) 10 mM, pH = 7.5) to a final concentration of 1.4×10^{11} NP/mL (i.e. 55 times less than the original concentration).

Nanoparticle characterisation. AuNPs were imaged using a transmission electron microscope (TEM, JEOL 2100, Japan) at an accelerating voltage of 200 kV. The samples were prepared by placing a drop (4 µL) onto ultrathin Formvar-coated 200-mesh copper grids (Tedpella Inc.), followed by drying in air at room temperature. For each sample, the size of at least 100 particles was measured to obtain the average and the size distribution. Digital images were analysed with the ImageJ software, using a custom macro to perform smoothing (3×3 or 5×5 median filter), a manual global threshold and the automatic particle analysis of ImageJ. The programme can be downloaded from <http://code.google.com/p/psa-macro>. A circularity filter of 0.8 was used to exclude agglomerates that could occur during drying.

UV-vis adsorption spectra were recorded with an Evolution 300 UV-vis spectrophotometer (Thermo Scientific, USA) at room temperature.

DLS and Zeta-potential measurements were obtained using a Zetasizer Nano ZS instrument (Malvern Instruments, UK). Hydrodynamic diameters were calculated using the internal software analysis from the DLS intensity-weighted particle size distribution.

Differential centrifugal sedimentation. DCS measurements (instrument model DC24000UHR, DCS Instruments Inc, USA) were performed in an 8–24 wt% sucrose density gradient with a disc speed of 22,000 rpm. Each sample injection of 100 µL was preceded by a calibration step using certified polyvinyl chloride (PVC) particle size standards with a weight mean size of 280 nm.

Contact angle. The contact angle with water was measured using a Digidrop Contact Angle metre (GBX, France). Contact angles of the differently functionalised AuNPs were measured by spotting a drop of sample solution (10 µL) on a silicon surface to form of confluent film. Then, a 0.5 µL water droplet was deposited onto this film and the contact angle was measured.

Contact angles were also measured on flat Au-surfaces functionalised with PEO-ligand. Au deposition on silicon wafers (3 min, ~100 nm Au) was performed by physical vapour deposition. The surfaces were cleaned with sonication in EtOH, followed by several washing steps with EtOH and MilliQ H₂O. They were then functionalised using 10 mM aqueous solution of PEO-ligand, and dried. A 0.5 µL droplet of either water or 1-bromonaphtalene was deposited on the surface and the contact angle measured.

For measurements of contact angles of flat SiO₂-surfaces with hydrophobic ligands, silicon wafers were treated with O₂ plasma (treatment time = 5 min) and then exposed to 1 mM aqueous solution of GPTMS at room temperature for 24 h. After washing with MilliQ H₂O, wafers were exposed to 2.24 mM solutions in water of propylamine, butylamine and dodecylamine at pH = 9 for 24 h at room

temperature before further washing with MilliQ H₂O. After drying, a 0.5 µL water droplet was deposited on the surface and the contact angle measured.

SDS-PAGE gel electrophoresis. AuNPs of 60 nm diameter, both pristine and PEO-functionalised were incubated with human serum (Sigma-Aldrich) for 24 h at 37 °C. The mixture was centrifuged (10,000 rcf, 5 min, 4 °C) and the supernatant carefully removed. The NP pellet was subsequently washed with 1× PBS (Gibco). This washing procedure was repeated three times. The final pellet was suspended in 20 µL Pierce Lane Marker reducing sample buffer (ThermoFisher) and incubated at 95 °C for 5 min. After a short spin down, the supernatant was loaded in 12% SDS polyacrylamide gel and run at 110 V, 25 mA in 1× SDS running buffer. After electrophoresis, the gel was Coomassie stained. Scanned gel images were analysed with the ImageJ software using sharp + smooth + brightness/contrast adjustment, followed by splitting of colour channels (best contrast for Red and Green). Quantification of the proteins was made by calculating pixel intensity in the central band (lower intensity = dark = more proteins) and plotted as the inverse of the pixel intensity.

Nanoparticles adsorption study by dark-field microscopy. Dark field microscopy (DFM) videos were recorded to measure the NP adsorption rates on the different collectors. Image analysis was performed using the open source graphics software ImageJ (<http://rsb.info.nih.gov/ij/>). A special data processing protocol was developed to enable automatic frame-by-frame analysis of the collector surface coverage by the NPs. The coverage boundaries were identified as red and green stains on a black background and only those with a size between 7 and 200 pixel units and circularity value between 0.20 and 1.00 were taken into account in calculating the degree of surface coverage (using the Analyse Particles function of ImageJ). After background correction, noise reduction (Despeckle, Kalmann Stack Filter with values 0.05–0.80), brightness, contrast and colour balance adjustment, the Unsharp Mask filter (radius 4.0 and mask weight 0.7) was applied. The image was then converted to an 8-bit file, this being required to adjust the threshold and analyse the particles with the provided function. This procedure allowed real-time kinetics analysis with a microfluidic chip, calculating the coverage degree of each substrate as a function of time. The association rate could then be determined, providing a quantitative measurement of the affinity of the NPs for the different collectors. These real-time measurements were carried out in static mode by using commercial slides provided by Ibidi (Sticky-Slide IV, Germany) with a channel volume of 30 µL and cell height of 0.4 mm. The channel was filled with the NPs solution using a syringe and the subsequent analysis of the collector surface was done with DFM.

Numerical solver. The Solver of Microsoft Excel[®] was used for finding the numerical solution of a system of non-linear equations using the GRG nonlinear solving method. The equations and the boundaries chosen are described in the results and discussion section, since they are part of the developed method.

Data availability

The authors declare that the data supporting the findings of this study are available from the corresponding authors on reasonable request.

Received: 20 March 2018 Accepted: 9 August 2018

Published online: 06 September 2018

References

1. Vance, M. E. et al. Nanotechnology in the real world: redeveloping the nanomaterial consumer products inventory. *Beilstein J. Nanotechnol.* **6**, 1769–1780 (2015).
2. Gessner, A. et al. Nanoparticles with decreasing surface hydrophobicities: influence on plasma protein adsorption. *Int. J. Pharm.* **196**, 245–249 (2000).
3. Shima, F., Akagi, T., Uto, T. & Akashi, M. Manipulating the antigen-specific immune response by the hydrophobicity of amphiphilic poly(γ-glutamic acid) nanoparticles. *Biomaterials* **34**, 9709–9716 (2013).
4. Chompoosor, A. et al. The role of surface functionality on acute cytotoxicity, ROS generation and DNA damage by cationic gold nanoparticles. *Small* **6**, 2246–2249 (2010).
5. Zhu, Z. J. et al. The interplay of monolayer structure and serum protein interactions on the cellular uptake of gold nanoparticles. *Small* **8**, 2659–2663 (2012).
6. Moyano, D. F. et al. Nanoparticle hydrophobicity dictates immune response. *J. Am. Chem. Soc.* **134**, 3965–3967 (2012).
7. Saha, K., Moyano, D. F. & Rotello, V. M. Protein coronas suppress the hemolytic activity of hydrophilic and hydrophobic nanoparticles. *Mater. Horiz.* **1**, 102–105 (2014).

8. Jones, M. C. et al. Quantitative assessment of nanoparticle surface hydrophobicity and its influence on pulmonary biocompatibility. *J. Control Release* **183**, 94–104 (2014).
9. Xiao, Y. & Wiesner, M. R. Characterization of surface hydrophobicity of engineered nanoparticles. *J. Hazard. Mater.* **215–216**, 146–151 (2012).
10. Doktorovova, S. et al. Modified Rose Bengal assay for surface hydrophobicity evaluation of cationic solid lipid nanoparticles (cSLN). *Eur. J. Pharm. Sci.* **45**, 606–612 (2012).
11. Carstensen, H., Müller, B. W. & Müller, R. H. Adsorption of ethoxylated surfactants on nanoparticles. I. Characterization by hydrophobic interaction chromatography. *Int. J. Pharm.* **67**, 29–37 (1991).
12. Desmet, C. et al. Characterisation of nanomaterial hydrophobicity using engineered surfaces. *J. Nanopart. Res.* **19**, 117 (2017).
13. van Oss, C. J. Acid–base interfacial interactions in aqueous media. *Colloids Surf. A: Physicochem. Eng. Asp.* **78**, 1–49 (1993).
14. Vallotton, P., Angel, B., McCall, M., Osmond, M. & Kirby, J. Imaging nanoparticle–algae interactions in three dimensions using Cytoviva microscopy. *J. Microsc.* **257**, 166–169 (2015).
15. Block, S., Fast, B. J., Lundgren, A., Zhdanov, V. P. & Höök, F. Two-dimensional flow nanometry of biological nanoparticles for accurate determination of their size and emission intensity. *Nat. Commun.* **7**, 12956 (2016).
16. Voitchovsky, K., Kuna, J. J., Contera, S. A., Tosatti, E. & Stellacci, F. Direct mapping of the solid–liquid adhesion energy with subnanometre resolution. *Nat. Nanotechnol.* **5**, 401–405 (2010).
17. Owens, D. K. & Wendt, R. Estimation of the surface free energy of polymers. *J. Appl. Polym. Sci.* **13**, 1741–1747 (1969).
18. Fowkes, F. M., Kaczinski, M. B. & Dwight, D. W. Characterization of polymer surface sites with contact angles of test solutions. I. Phenol and iodine adsorption from methylene iodide onto PMMA films. *Langmuir* **7**, 2464–2470 (1991).
19. Köstler, S., Delgado, A. V. & Ribitsch, V. Surface thermodynamic properties of polyelectrolyte multilayers. *J. Colloid Interface Sci.* **286**, 339–348 (2005).
20. Capomaccio, R. et al. Determination of the structure and morphology of gold nanoparticle–HSA protein complexes. *Nanoscale* **7**, 17653–17657 (2015).
21. Sellers, H., Ulman, A., Shnidman, Y. & Eilers, J. E. Structure and binding of alkanethiolates on gold and silver surfaces: implications for self-assembled monolayers. *J. Am. Chem. Soc.* **115**, 9389–9401 (1993).
22. Oćwieja, M., Morga, M. & Adamczyk, Z. Self-assembled silver nanoparticles monolayers on mica-AFM, SEM, and electrokinetic characteristics. *J. Nanopart. Res.* **15**, 1460–1465 (2013).
23. van Oss, C. J. *Interfacial Forces in Aqueous Media. 2nd Edition.* (CRC Press, Boca Raton, Florida, USA, 2006).
24. Wood, J. A. & Rehmann, L. Geometric effects on non-DLVO forces: relevance for nanosystems. *Langmuir* **30**, 4623–4632 (2014).
25. Turkevich, J., Stevenson, P. C. & Hillier, J. A study of the nucleation and growth processes in the synthesis of colloidal gold. *Discuss. Faraday Soc.* **11**, 55–75 (1951).
26. Liu, X., Atwater, M., Wang, J. & Huo, Q. Extinction coefficient of gold nanoparticles with different sizes and different capping ligands. *Colloids Surf. B Biointerfaces* **58**, 3–7 (2007).
27. Ojea-Jiménez, I. et al. Highly flexible platform for tuning surface properties of silica nanoparticles and monitoring their biological interaction. *ACS Appl. Mater. Interfaces* **8**, 4838–4850 (2016).

Acknowledgements

The fruitful discussions with H. Lyklema and H. van Leeuwen, from University of Wageningen, are gratefully acknowledged. The authors thank N. Gibson and D. Gilliland for the proofreading of the revised paper.

Author contributions

A.V. conceived the method, designed the experiments, performed AFM characterisation of collectors, conceived the numerical extrapolation of the nanoparticles hydrophobicity, and wrote the manuscript. C.D. produced and characterised the collectors. I.O.J. synthesised functionalized and characterised Au and Si nanoparticles. A.O. developed the real-time automatic detection of particles by combination of DF microscopy and ImageJ. R.C. performed analysis of DCS measurements. F.R. conceived the method, wrote and revised the manuscript. P.C. performed data analysis for the characterisation of NPs by DCS/DLS combination, participated in the design of the experiments, wrote and revised the manuscript.

Additional information

Supplementary information accompanies this paper at <https://doi.org/10.1038/s42004-018-0054-7>.

Competing interests: A.V., C.D., F.R. and P.C. declare competing interests since they are inventors of the patent WO2016177641 with application date 16/11/2016. The remaining authors do not declare any competing interest.

Reprints and permission information is available online at <http://npg.nature.com/reprintsandpermissions/>

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2018