Back to basic, optical evaluation of motion in electric fields for specific surface charge determinations

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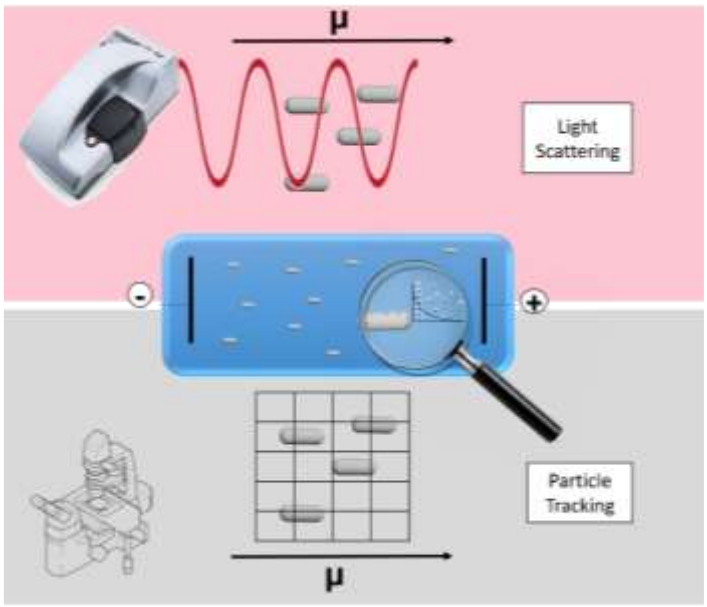

ABSTRACT

Dynamic Light Scattering (DLS) is a widely used tool for the measurement of Zeta Potential, taking benefit of automated detection to achieve fast measurements. However, the mathematical criteria on which the calculations in DLS devices are based, imply a very narrow set of conditions. One of them is a perfect spherical shape, since a hard sphere model is assumed for calculating scattering patterns and therefore the analysis of different shapes could result in significant deviations. One frequent example where the determination of the surface charge in rod-shaped colloids is required, is the characterization of bacterial surface charge, which is complicated by complex surface properties. To test whether the commercial device gives a reasonable approximation, we constructed a homemade optical device and tested inorganic spherical and rod-shaped SiO_2 particles and compared them to a model bacterium. A different case is the determination of surface potentials of light sensitive materials under illumination. Commercial devices often do not allow the additional implementation of light sources other than the laser, but our setup flexibly enables us to plug in different illuminations.

KEYWORDS

Surface Charge, Electrophoretic Mobility, Rod-shaped particles, Bacteria, Zetameter and Dynamic Light Scattering

Introduction

Colloidal science has evolved in a plentitude of ways over the last century, starting from synthetic strategies such as the Stöber synthesis,[1] hot injection[2] or the polyol synthesis,[3] to the advances in instrumentation and characterization techniques like electron microscopy, spectroscopy or light scattering. Significant concepts to understand the properties of charged colloidal surfaces were developed since the nineteenth century.[4] H.v.Helmholtz himself introduced the idea of an electrical double layer (EDL). EDL refers to the ionic environment surrounding a charged colloidal particle, which consists of hydrated and bare ions of mostly opposite charge, neutralizing the charged groups on the colloid's surface. In the 1930's, this concept was refined by L.G.Gouy and D.L.Chapman, including the concept of a diffuse double layer around the particle, as well as the influence of steric effects.

The EDL consists firstly of a layer where bare ions (Stern layer), followed by a layer of hydrated ions, are strongly bounded to the colloid surface. Beyond this layer, the diffuse layer is found. There, the hydrated ions are less firmly associated to the colloid and the ion concentrations depart from the electroneutrality values.[5,6] The diffuse layer depends on factors as the pH and ionic strength; it is formed by ions with the same and opposite charge of the colloid's surface.[7]

The EDL is a dynamic construct and therefore does not lead the colloid to have zero net charge, which becomes obvious when an electric field is applied to the colloidal suspension. The colloids then move towards the electrode with opposite charge; i.e. electrophoresis.[7,8] Thus, within the diffuse layer a hypothetical shear/slipping plane or boundary exists, inside of which the ions move in the same direction as the charged colloid; meanwhile the ions outside this boundary zone migrate as the dispersing fluid.[5] These interactions lead to the presence of an electrokinetic potential in the shear plane, known as Zeta Potential (ζ).

Due to this definition, Zeta Potentials are difficult to measure directly but can be calculated by the evaluation of the electrophoretic mobility (μ) of particles in an electric field. According to the Henry Equation

$$\mu_e = \frac{2\varepsilon\zeta f_{(k\alpha)}}{3\eta} \qquad (1)$$

the electrophoretic mobility depends on the viscosity η of the dispersion media (in cP), ε the dielectric constant and the Henry's function $f_{(k\alpha)}$, a parameter depending on the ratio of the particle radius to the thickness of the EDL. Here, it becomes obvious that the shape of the colloid is of pivotal importance, for example, the frequently used Helmholtz-Smoluchowski approximation gives $f_{(k\alpha)}$ a value of 1.5; considering a double layer which is thin compared with the particle radius with a spherical geometry[9] (Equation 2)

$$\mu_e = \frac{\varepsilon\zeta}{\eta} \qquad (2)$$

Since the Zeta Potential is used as indicator of the stability of a colloidal system[10,11] there was a great interest in the development of electrophoresis based techniques with optical evaluation (Particle electrophoresis, Zetameter setup).[12,13] With the introduction of an automated detection based on laser scattering methods (Dynamic Light scattering, DLS)[14] the evaluation of Zeta Potentials became frequently used as screening method of production processes. More recently, commercial devices were also improved to allow measurements on biological molecules with low scattering intensities and therefore, moved into the focus of

biologists.[15–17] Lately, the interest in characterizing the surface of bacteria as biological colloids has grown due to different applications in biofilms,[18–20] biotechnological advances[21] and biohybrid micromotors.[22,23]

Given this interest, microbiologists evaluate the charge of bacteria by a qualitative method based on electrostatic interactions of the bacterial surface with strongly charged fluorescent biomolecules. Thus, alterations in the overall charge of the cell (environmentally induced or due to mutations) can be screened by analyzing the capability to bind negatively or positively charged proteins.[24] Despite the unknown nature of interaction, this method gives reasonable insights as long as all other factors are kept constant. This limit makes evident the need of other strategies for bacteria surface characterization. Most of these characterization techniques are based on the electrophoretic/electrostatic features of bacteria: microelectrophoresis, electrostatic interaction chromatography, aqueous two phase partitioning, isoelectric equilibrium analysis, and among others, electrophoretic DLS.[13]

The general aim of this study is to determine how different conditions and shapes affect the electrophoretic mobility of colloids using a Zetameter setup; and compare the obtained values with the widely used DLS method. To do so, we construct a home-made electrophoresis setup (Zetameter), in which the calculations of electrophoretic mobility are based on direct observation of particles moving under an applied electric field. The analysis of different particles by both methods, evidenced agreement among the Zeta Potential values obtained for spherical silica particles; but significant differences were found for the values rod-shaped silica particles. These findings are compared to the equivalent results of bacteria as rod-shaped living particles with different physicochemical properties. Thus, we used the Zetameter to calculate the electrophoretic mobility of a *Lactobacillus* strain used for the preparation of "Sauerkraut"; as an alternative methodology; considering that the deviation from the typical spherical shape could result in laser scattering effects in DLS measurements. In addition, we show the feasible application of our Zetameter setup to analyze particles with light induced activity, like spherical TiO_2, particles, as an example of direct observation of light induced phenomena, which is not possible in the DLS approach.

Results and Discussion

There are plenty of aspects to compare both methods, obvious are the ease of use, the cost of equipment, training necessity, adaptability of the method among others. Due to single use cells, DLS has clear advantage over the Zetameter in terms of handling the samples. Concerning the analytics, the Zetasizer device uses dynamic light scattering to evaluate the particle speed. For this method, a monochromatic light source (usually a laser) is required. When the laser light hits a small particle, the light is scattered. These scatter patterns are dependent on the size ~~(~~and shape~~)~~ of the particle, which is accounted for in the mathematical model. Since the particles are moving, the signals change dynamically and also interact with the scattered light of the surrounding colloids, leading to interference in the signals. The detected signal fluctuations are transformed into an autocorrelation function, which is further processed to extract more user friendly data values. In the Zetameter instead, Particle Tracking Analysis (PTA), is used to obtain particle speed data. In this simple and reliable method, the time resolved trajectories are tracked from videomicroscopy, reducing the system to a two dimensional electrophoretic one, causing the loss of the actual 3D movement of the particles.[25] Particles analyzed by PTA must have stronger scattering than the background (which is the case of bacteria); leading to a lower density of results than in DLS. While the

asymmetric scattering is especially disturbing for increased sizes in the DLS, the problem of the settling down of large particles is critical in both methods. One advantage of the more laborious Zetameter strategy is the feasibility of *in situ* observation of kinetic phenomena as assembly of particles, the analysis of fluorescent particles without costly equipment or the application of special irradiations. However, when in DLS heavy particles are removed from the observation fields by sedimentation, in the Zetameter the osmotic influence in vicinity of the wall can be rarely avoided for particles with a density mismatch.

The Zeta Potential values are obtained using **Equation 2** in the Zetameter methodology. As previously stated, the suitability of the Helmholtz-Smoluchowski approximation is based on a spherical shape and a thin EDL in comparison with the particle radii.[5]

Evaluation of Silica particles

Both, SiO_2 spherical and rod-shaped particles were synthesized by previously established procedures.[26,27] The size and shape of the silica rods were tuned to be comparable to the bacteria (see Figure 1). In the case of raw silica spherical particles, the ζ values obtained by Zetameter and DLS (-33±4mV and -34±3mV; respectively) are shown in Figure 2A. T-test revealed that they are not statistically different. Nevertheless, the values for rod-shaped particles presented in Figure 2B (-23±1mV and -26±3mV) show a statistically significant difference when T-test was carried out.

In order to demonstrate how surface functionalization can influence the Zeta Potential calculations, Silica particles were functionalized via two different strategies to obtain a coverage of the surface with amino and thiol groups, both binding readily to Au surfaces. Subsequently, we attached or grew gold nanoparticles (Au-NPs) on the surface (see Figure 1B, 1D and 1E and Supplementary Information 1, SI1). Thus, we changed not only the chemical and charge properties, but also modified the optical properties of the composites, which influence the scattering patterns and intensities of the laser (Refractive Index (RI) SiO_2: 1.5534[28] RI Bacteria: ~ 1.38[29] RI Au: 0.58[30] - 0.88[31]).

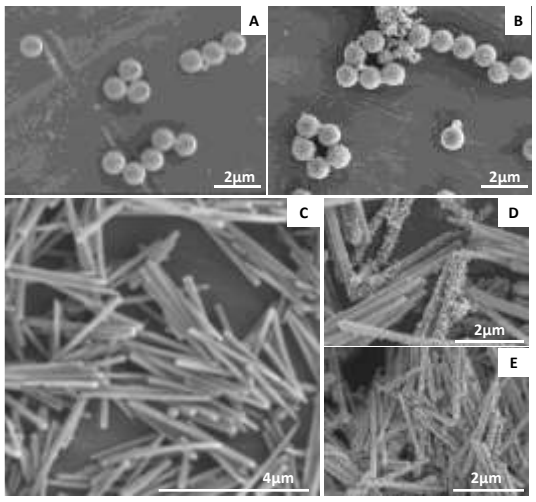

Figure 1: SEM micrographs of A) 1µm SiO_2 and B) Au@SiO_2–NH_2 spheres. C) SiO_2 rods, D) Au@ SiO_2– NH_2 rods and E) Au@SiO_2 -SH rods

Au-NPs grown on the amine functionalized particles (Au@SiO_2 –NH_2 hard sphere and Au@SiO_2 rods-NH_2, Figure 1B and 1D), are slightly smaller than the ones attached to the

thiolated particles (Au@SiO_2-SH), as seen in Figure 1E. This size difference owes to the different synthetic methods (*in situ* growth versus attachment), but does not affect the study.
In comparison to the plain spheres, the potential values obtained by the determination of electrophoretic mobility by Zetameter setup and DLS for the Au@SiO_2 particles are not significantly different (+8±1mV and +6±1mV). The presence of the Au-NPs on the surface of the silica spheres seems not to produce deviations (see Figure 2A and SI2).
In agreement with the abovementioned results for the plain silica rods, the differences between the methods are even larger in the case of Au@SiO_2 rods (see Figure 2B and SI2). The most pronounced difference is seen for Au@SiO_2- SH rods (-9±1mV and -20±2mV). However, despite the fact that we observed particles with different surface properties, the migration of all particles seems to be governed mainly by a hydrodynamically favorable orientation (the rod-shaped particle in line with the flow, see Figure SI5). The shape caused a clear deviation when comparing the data obtained by Zetameter with the DLS data. T-tests revealed significantly different values for all analyzed samples. Since the Zeta Potential is an artificial value, we have no direct way of its determination, but in line with our theoretical assumptions, we find that a rod-shaped geometry seems to put clear limits to the application of DLS.

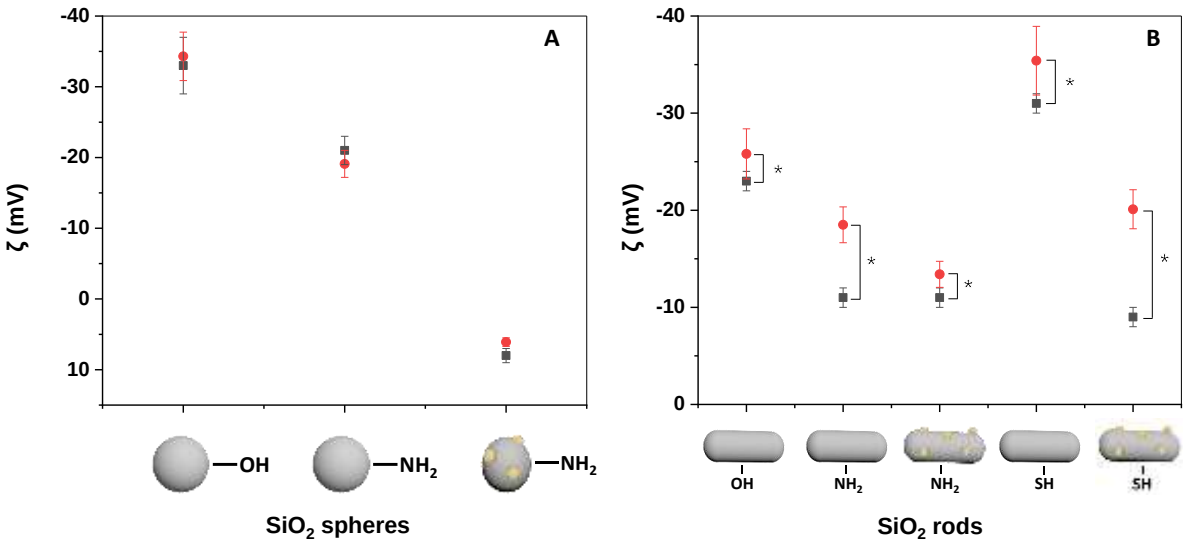

Figure 2: Comparison of the mean Zeta Potential and standard deviations values obtained using electrophoretic mobility (black squares, at 40V) and dynamic light scattering (red squares) of SiO_2 spherical (A) and SiO_2 rod particles (B) Statistical different values: * P ≤ 0.05

As expected, the DLS and Zetameter results have good agreement for spherical particles. Both techniques can be used complimentarily and the laser scattering gives reliable measurements, for which the DLS has replaced manual measurements for most laboratory uses. Also concerning the inorganic rod-shaped particles we confirm the expected deviations, leading to values that are significantly different for plain, functionalized and Au-composites.

Evaluation of Bacterial cells

In order *measure and* calculate the Zeta Potential of a real rod-shaped living particle we chose a type of Lactobacillus, autochthonous to the German culinary culture (see Experimental Section). This bacterium has a pronounced rod-shaped geometry (see Figure 3), no flagella that could lead to additional drag effects [37] and it is a non-motile species; so not overlaying movements are to be expected. T-test showed significant difference in Zeta Potential values

of *Lactobacillus* strain, obtained by the Zetameter and DLS methodologies (-14±3mV and -11±2mV, respectively; see SI3). These deviations can be attributed to its elongated shape and agree with the abovementioned differences for the rod silica particles. As for silica rods, the bacteria orientation during the electrophoretic assays is hydrodynamically favorable, i.e. in line with the flow (see SI5). What apparently does not play an important role is the soft and porous structure of the Bacterial surface. However, to evaluate the influence on the surface slip and therefore the hydrodynamics in vicinity of the particles and the electrophoretic mobility, more controlled modifications of bacterial morphologies and surface properties would be needed. Also additional shape deviations caused by substructures of the bacterial surface such as pili, or fimbrils are not considered, even though they are expected to have a rather important influence on the drag.[37]

Further deviations between the values obtained can be due to the structural differences of the bacteria wall. In general, cell surfaces are highly dynamic and respond strongly to external *stimuli*, by adsorptions of ions and others substances, altering the surface properties.[33] Even though the morphology and size of the studied silica rods and Lactobacillus are similar (see SEM insets in figure 3B), the distribution of charged groups on bacterial surfaces may not be homogeneous, [34] making the bacterial surface chemically and structurally more complex than the one inorganic colloidal particles, affecting the EDL interactions (Schematically presented in Figure 3B).[35,36]

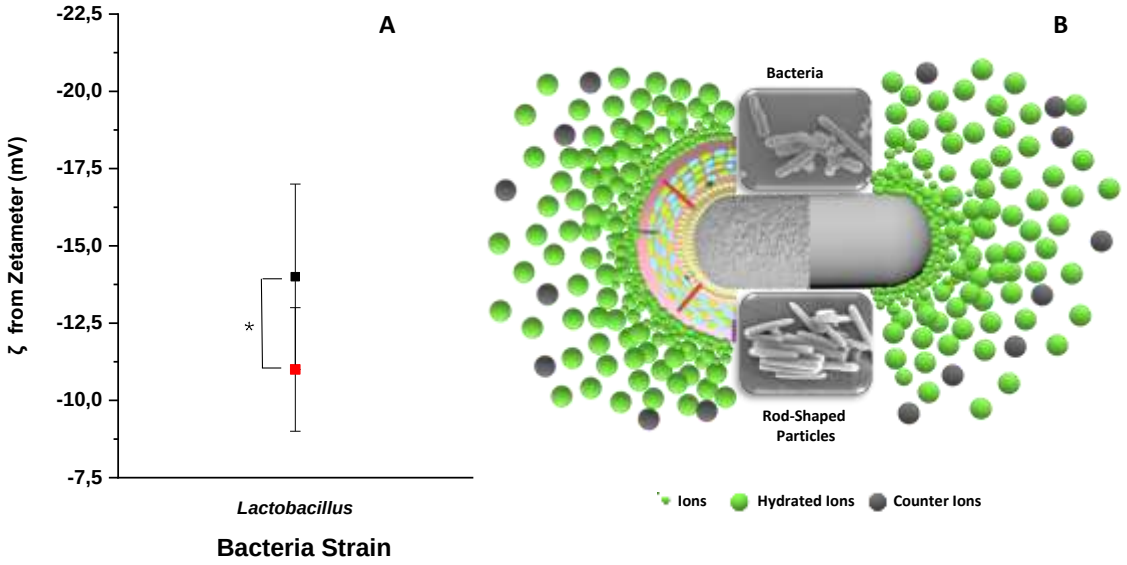

Figure 3: Comparison of the mean Zeta Potential and standard deviations values obtained using electrophoretic mobility (black squares, at 40V) and dynamic light scattering (red squares) of Lactobacillus (A) and schematic representation of the differences of the EDL between bacteria and rod particles (B). Statistical different values: * $P \leq 0.05$

These biologically specific restrictions combined with the limitations of DLS for the measurement of complex shapes; show the importance of the comparison of both strategies, to evaluate their suitability for the analysis of bacteria surface. Despite the calculated statistically significant deviations between both methods, the obtained absolute and relative differences between the DLS and the Zetameter results are small and for most biological applications probably not of major importance.

Evaluation of the influence of illumination on the electrophoretic mobility of photoactive particles

One of the major drawbacks of automated DLS devices is the relative lack of flexibility of in terms of added experimental conditions. In order to evaluate the surface properties of active photocatalytic or photoswitchable materials, *in situ* illumination is required, which is not implementable in most commercial devices. Here, our setup connected to an optical microscope is an excellent way to evaluate the changes that these particles undergo. To evaluate these changes, we chose simple spherical TiO_2 particles of a mixed phase rutile anatase, which have shown a low photoinduced activity in water.[37]

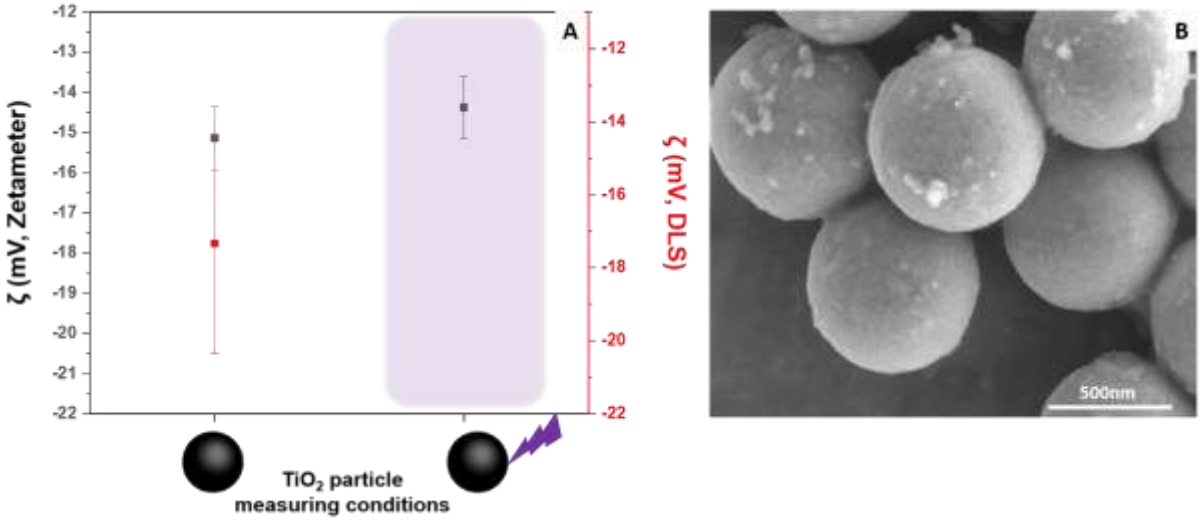

Figure 4: Evaluation of the mean Zeta Potential and standard deviations values obtained using DLS in red squares and the Zetameter setup at 40V in black squares (A) and SEM image of the titania particles (B)

In Figure 4 we see the different values obtained for titania particles in water (check SI4 for values). The deviation for non UV illuminated particles is rather large considering their perfect spherical shape, which could be due to the influence of the laser, or alternatively, due to the small part of UV light present in the microscope illumination.

UV light in water seems to decrease the potential slightly, but no pronounced effect can be observed, which is coherent with the low activity we found for these particles. However, the method gave access to an interesting data point, that we could not obtain using a commercial DLS setup. Further interesting data is expected by using different media (e.g. H_2O_2) as well as other, maybe switchable particles.

Conclusions

We evaluated the suitability of two different methodologies (DLS and Zetameter) for the determination of Zeta Potential values of different shapes of colloids. The comparison between the values calculated from both strategies resulted similar values in the case of spherical silica particles. On the contrary, all analyzed rod-shaped particles showed the expected deviations in the DLS measurements due to their distinct rod shapes. In case of bacterial cell, the

deviations from spherical shape impact the DLS values, but the deviations are rather small, so that the values can give a reasonable approximation. However, no information about the surface morphology can be obtained, despite the fact that it would affect directly the hydrodynamics and the electrophoretic motion. Since it would be present in DLS as well as the Zetameter, no conclusions can be drawn. On the other side, this regained method can become a powerful tool evaluating colloids under different illumination conditions, as was proven by the evaluation of the titania particles.

Experimental Section

Materials and reagents:

Tetraethoxysilane (TEOS), (3-Aminopropyl) triethoxysilane (APTES), Ammonia (NH_3, aq. 25%), Gold (III) chloride trihydrate ($HAuCl_4 \cdot 3H_2O$), 1-Pentanol, 3-Mercaptopropyltrimethoxysilane (MPTMS), sodium citrate, trisodium citrate, Polyvinylpyrrolidone(PVP; Molar weight: 40K) were purchased from Sigma Aldrich, of analytical grade and used as received without further treatment. Ethanol (EtOH, absolute) was purchased from VWR and potassium chloride from J.T. Baker. Titanium (IV) iso-propoxide (TTIP) was purchased from Alfa Aesar Co. Ltd. Dodecylamine (DDA) was obtained from Fluka. Milli-Q water was used for preparing aqueous solutions

Preparation and functionalization of 1 µm SiO_2 spherical particles:
1.9 ml TEOS were diluted in 13 ml isopropanol and mixed with 0.7 ml NH_3 (25%) in 1.4 ml H_2O. The solution was stirred at room temperature and aged for 60 min at 50°C to obtain seeds. A solution of 7 ml TEOS in 300 ml propanol was added to the seed solution under constant stirring. Subsequently 20 ml NH_3 (25%) in 40 ml of water were added and heated to 50°C. As last step 120 ml TEOS were added at an addition rate of 0.5 g/min. Several filtration and washing steps with water are required to remove NH_3 and solvent residues.

Preparation and functionalization of SiO_2 rods:
As described somewhere else,[26] the preparation of 2-3µm SiO_2 rods requires a solution of 1g of PVP in 10 ml of 1-pentanol. Then, 1 ml of ethanol, 340µl of water and 80µl of sodium citrate (0.18 M) were added and stirred for 5min. Then, 200µl of ammonia and 60µl of TEOS were added. The solution was stirred for 5 min. and then left under static conditions for 18h. The obtained rods were washed several times with ethanol and separated by centrifugation.

Amine ($-NH_2$) or thiol (-SH) Functionalization of SiO_2 spheres or rods:
100 mg of SiO_2 particles or rods were dispersed in 5mL of EtOH; then 150µl of APTES (for amine functionalization) or 150µl of MPTMS (for thiol functionalization) and 300µl of ammonia were added. The particle suspension were stirred overnight. The washing procedure included centrifugation and rinse several times with ethanol.
Amine groups ($-NH_2$) have partial positive charge, making that APTES functionalized particles have less negative surface charge as shown in SI2 Table I. Thus, the synthesis of Au NPs with the syringe pump approach is driven firstly by the immobilization of the Au NPs precursor ($AuCl_4^-$) and then the reducing stage with the ascorbic acid and trisodium citrate. The process is favored due to the known affinity of gold nanoparticles to amine moieties.[38]
Thiol groups (-SH) maintain the overall negative charge of the raw particles.[38,39] Chemical affinity of Au with thiol moieties leads to the attachment of previously synthesized particles to

thiolated surfaces.[38,39] Moreover, it is favored due to the positive ζ of the Au NPs as shown in SI 2 Table I.

Preparation of Au@amine functionalized SiO_2 particles or rods.

A seed growth approach to obtain uniform gold nanoparticles was adapted as follows.[40] In a three necked flask, containing a suspension 25mg of the SiO_2 particles or rods in 20ml of water was stirred vigorously. Simultaneously, two solutions: A (8 ml H $[AuCl_4^-]$ (1%) + 2 ml water) and B (2 ml ascorbic acid (1%) + 1 ml trisodium citrate (1%) + 7 ml water) were drop-wise with syringe pump at a rate of $20ml \cdot h^{-1}$. The resulting suspension was boiled for 30min; and the particles were separated by centrifugation and washed several times with water.

Preparation of Au@thiol functionalized SiO_2 rods:

The preparation of Au was adapted from somewhere else.[41] 10ml of tetralin, 10ml of Oleylamine(OAm) and 0.1g of $HAuCl_4 \cdot 3H_2O$ were combined and magnetically stirred under N_2 flow for 10 min. Then, a solution 0.5 mmol of Tetrabutylammonium bromide (TBAB), 1ml of tetralin and 1mL of OAm (mixed by sonication) were injected into the abovementioned solution. The mixture changed to a deep purple and left to react for 1h. The nanoparticles were precipitated by adding 60ml of ethanol, and then washed with ethanol and separated by centrifugation. These Au NPs (with a Zeta Potential value of +31mV) were dispersed in 5ml of chloroform. 25mg of the thiolated SiO_2 rods were suspended in 3ml of ethanol. Then, 3ml of suspension of gold nanoparticles in chloroform were added. The mixture was shaken overnight and separated by centrifugation followed by washing steps with ethanol.

Preparation of TiO_2 particles

The synthesis is based on the hydrolysis and condensation reaction of TTIP[42,43]: 0.18 ml of water was added to mixture solution containing 105 ml of methanol and 45 ml of acetonitrile. Then 0.28 g of DDA was dissolved in the mixture under stirring. After stirring for 10 min, 1 ml of TTIP was added drop wisely and stirred for 12 h. DDA acted as a catalyst and molecular template to introduce the pores into the TiO_2 particles. Then the particles were washed with methanol three times and dried at 80°C. The particles were heated in tubular furnace under nitrogen flow for 2h at 600°C.

Preparation of *Lactobacillus*

Autochthonous Lactobacillus from cabbage (*Brassica oleracea*) was cultivated by a traditional recipe: the cabbage was sliced into thin stripes, about 5 wt% of salt were added and the mixture was pounded until liquid emerged from the vegetable and covered the mixture. Then the mixture was covered with leafs and left fermenting at about 27°C for seven days. After that, the mixture was transferred to a cool place and left maturating for several weeks prior to use. Before characterization, the bacteria were centrifuged, washed and prepared for the individual characterization steps.

Electron Microscopy Characterization

Scanning electron microscopy (SEM) was performed on Aluminum covered holders in a Zeiss DSM 982 Gemini instrument. Bacteria samples were washed three times and collected after centrifugation at 3000rpm, and resuspended in 3% glutaraldehyde solution. Then, silicon wafers were incubated with bacteria suspension for 30min. Afterwards, bacteria were dehydrated in a series of increasing aqueous ethanol concentrations (30%, 50%, 60%, 75%, 90% and 100%) for 5 min in each solution. The same procedure was repeated for the same

concentrations and times for Hexamethyldisilazane solutions. The samples were then dried overnight.

Determination of Zeta Potential by means of DLS

A Malvern Zetasizer® Nano ZSP with a10mW red laser (632.8 nm) was used to record the Zeta Potential of the different type of silica particles and bacteria. In each case, the sample was equilibrated for 1 min at 25 °C before starting the measurements. Three measurements, each consisting of 30 runs were performed for each sample.

Determination of Zeta Potential by means of electrophoretic mobility via a Zetameter set-up:

An electrophoretic cell was filled with the particle suspension to be studied. Thus, two Pt mesh electrodes plugged to a power source, were immersed in the dispersion and then; increasing voltages (from 10V to 50V) were applied. To avoid bubble formation on the electrodes, their polarity were changed and then decreasing voltages (from 50V to 10V) were applied. Conductivity was kept to $1{,}411 \times 10^{-3}$ $S \cdot cm^{-1}$ value using KCl 0.01M for inorganic particles and with the appropriate dilutions of the bacteria suspensions. Electrophoretic mobility of bacteria was carried out analyzing at least 90 bacteria cell per voltage. An analogue procedure was used for the inorganic counterparts. The electrophoretic movement of the particles were recorded with an Inversed Optical microscope (Carl Zeiss Microscopy GmbH Germany) equipped with a Zeiss Colibri lamp; using an acquisition time of 40ms and a 50x long working distance objective (Zeiss® LD EC Epiplan-NEOFLUAR 50x/0.55 DIC 60). The analysis of the recorded videos was carried out using tracking tool from ImageJ software version 1.52i (National Institute of Health, USA)[44]

T-test: statistically significant differences in mean ζ for all SiO_2 rods and *Lactobacillus* with 95% confidence interval.

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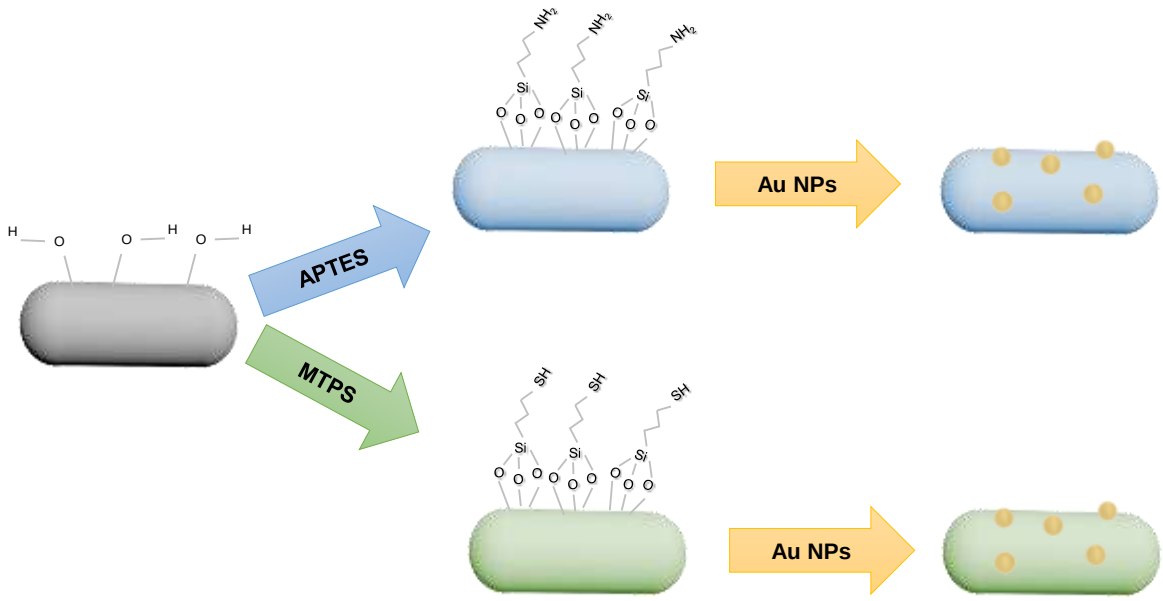
Supplementary Information (SI)

SI 1: Schematic summary of the chemical functionalization of silica rods.



SI 2: Table I: Summary results for the determination of Zeta Potential of SiO_2 sphere and rod particles by the two studied approaches. T-test: non-statistically significant differences with 95% confidence interval in mean ζ for spherical particles. Statistical different values for mean ζ of silica rods.

Particle type	ζ from Zetameter (mV)	ζ from Zetasizer (mV)
SiO_2 hard sphere	-33±4	-34±3
SiO_2 –NH_2 hard sphere	-21±2	-19±2
Au@SiO_2 -NH_2 hard sphere	+8±1	6±1
SiO_2 rods	-23±1	-26±3
SiO_2 rods-NH_2	-11±1	-19±2
Au@SiO_2 rods- NH_2	-11±1	-13±2
SiO_2 rods-SH	-31±1	-35±4
Au@SiO_2 rods-SH	-9±1	-20±2
Au NPs	-	+31±2

SI 3: Table 2: Summary results for the determination of Zeta Potential of *Lactobacillus plantarum* by the studied approaches.

Sample	ζ from Zetameter (mV)	ζ from Zetasizer (mV)
Lactobacillus	-14±3	-11±2

SI 4: Table 3: Summary results for the determination of Zeta Potential of *Lactobacillus plantarum* by the studied approaches.

Sample	ζ from Zetameter (mV)	ζ from Zetasizer (mV)
TiO_2 particles	-15.1±0.8	-17.3±3
TiO_2 particles + UV	-14.4±0.8	-

SI 5: Screenshots of the particle tracking for the determination of the electrophoretic mobility of different rod and spherical SiO_2 particles (A,B), TiO_2 particles under UV-light (C) and bacteria (D) using a Zetameter set-up, with the corresponding inset for the type of motion that rod particles have through the application of the electric field. As it can be seen, inorganic and living rod particle present similar orientation; mostly aligned to the flow, with short fluctuations.