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# Characterization of biological liquids by Modulated 3D Cross-Correlation Dynamic Light Scattering

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**ABSTRACT**
The deep biomedical knowledge reached in the past decades in many fields of medicine and biology has been accompanied by continuous technological progress in measurement instrumentation and analysis techniques. This development is important also to improve the characterization and the understanding of the interactions between nanoparticles and biological liquids, due to emerging applications such as drug delivery, bioimaging, biosensing, diagnostics and photothermal therapy.
In this study, we characterize the interaction between mouse serum and gold nanoparticles (NPs) and nanorods (NRs) by Modulated 3D Cross-correlation Dynamic Light Scattering (DLS), in order to demonstrate that this technique can be applied to the investigation of complex biological liquids. The analysis of the size distribution of the hydrodynamic radius reveals three different contributions from particles motion, associated to rotation, translation and agglomerates. Moreover, we show that the interaction between Au NPs or NRs and mouse serum depends on the aspect ratio of the Au particles. These results are promising for deepening the knowledge on proteins-nanoparticles interaction, for laboratory-based experiments as well as for sensing and diagnostic applications and nanoparticles based medical therapy.
**Keywords**: dynamic light scattering, gold nanorods, gold nanoparticles, mouse serum, protein interaction, biomedical.

## 1. INTRODUCTION

In the biomedical field, it is important to use characterization methods that are not invasive and non-destructive. Modulated 3D Cross-Correlation Dynamic Light Scattering (DLS) is a recent non-destructive technique often used for nano- and microparticle size determination [1], within a range of 0.1 nm to 10 µm, even in turbid suspensions [2,3]. Due to these capabilities, in the last years DLS has been increasingly used also for the characterization of biological samples, such as proteins solutions [4,5].

Gold nanoparticles (Au NPs) and nanorods (NRs) are already widely studied by DLS [6,7] in order to monitor their size and homogeneity after synthesis processes. It has been shown that with this technique it is possible to analyse the contribution to the motion due to rotation of non-spherical particles [7], and that the obtained information can be used to determine the degree of the non-sphericity of particles. Along with the increasing importance of Au NPs in biomedical applications, i.e. drug delivery, bioimaging, biosensing and photothermal therapy [8,9], DLS is becoming a powerful method to study NPs interaction with proteins. To our knowledge, so far, well defined protein solutions have been taken into account for DLS studies, e.g. [4,7].

In this research, we focus our attention on the interaction between Au NPs and Au NRs of different dimensions with mouse serum. We demonstrate the successful application of DLS for determining the size distribution of components in such a complex system, and we show how the calculated hydrodynamic radii can be used to analyse the shape of agglomerates and the interaction between particles.

## 2. EXPERIMENTAL

### 2.1 Materials

Four different aqueous suspensions of gold NPs and NRs, all stabilized by bare citrate (purchased from NanoComposix), were used in the present work:

- Spherical NPs, with nominal diameter of 40nm (Au NPs 40nm), concentration of 0.05mg/mL;
- NRs with absorption peak at 660 nm, nominal length and diameter of 48 nm and 18 nm respectively, aspect ratio of 2.6 (Au NRs AR2.6), concentration of 31 µg/mL;
- NRs with absorption peak at 800nm, nominal length and diameter of 55 nm and 15 nm respectively, aspect ratio of 3.6 (Au NRs AR3.6), concentration of 16 µg/mL;
- NRs with absorption peak at 980nm, nominal length and diameter of 85 nm and 15 nm respectively, aspect ratio of 5.6 (Au NRs AR5.6), concentration of 18 µg/mL.

Mouse serum was purchased by BioWest, already sterile and filtered. In order to increase the scattering intensity in the DLS and to decrease the presence of large agglomerates additional treatments were performed. The mouse serum has been filtered through a membrane with pores of 1.2 µm diameter. Samples of different concentrations (33%, 44%, 50% and 60%) were prepared by dilution in ultra-pure DNase-free distilled water, followed by

centrifugation at 4000 rpm for 2 hours. The interaction of Au NPs and NRs with mouse serum was studied by adding 7% of NPs suspension (50 µL in 700 µL of serum). Glass cuvettes of 10 mm x 10 mm were used for DLS.

### 2.2 Modulated 3D cross-correlation dynamic light scattering

NP sizing by DLS is based on Rayleigh scattering, i.e. particles are supposed to be significantly smaller than laser wavelength, and on Brownian motion. The scattered intensity is measured and analysed; it decays exponentially, and its decay parameter is proportional to the translational diffusion coefficient, $D_t$. For spherical particles, it can be calculated by the Stokes-Einstein equation:

$$D_t = \frac{kT}{6\pi\eta R_H} \tag{1}$$

where $k$ is the Boltzmann constant, $T$ is the temperature, $\eta$ is the viscosity and $R_H$ the hydrodynamic radius.

Our system is equipped with an innovative setup (LS Instruments) that uses two laser beams, and two detectors, temporally isolated from each other, with the suppression of signal cross talk. Thanks to this configuration, we can measure even turbid samples, because multiple scattering is significantly reduced. The laser has a wavelength of 685 nm, the high-speed intensity modulators have a frequency of 5 MHz and the scattering angle is 90°.

All data reported in the present paper were acquired at a temperature of 20°C. We have recorded three repetitions of 40s per sample, and we have performed the analysis on the intensity size distribution by the CONTIN fit method [10], since our samples are mostly polydisperse. As a result, we show an average of the measured hydrodynamic radius with its standard deviation.

### 2.3 UV-vis-NIR spectroscopy

Optical absorbance spectra are recorded in the range from 200 nm and 1000 nm using an UV-vis-NIR spectrometer (OceanOptics Flame, cuvettes with 5mm path). Both deuterium and halogen lamp were used.

## 3. RESULTS AND DISCUSSION

### 3.1 Au NPs and mouse serum characterization

The optical properties of gold NPs and NRs have been characterized by absorbance spectroscopy. In Fig.1(a) a comparison of the different spectra is shown. For the Au NRs we can observe the two surface plasmon resonance (SPR) bands: the transversal one is around 510 nm, while the longitudinal one varies with the aspect ratio of the nanorods [11]. On the other hand, for Au NPs 40 nm, only the transversal band is present. The absorbance peaks are in agreement with the values reported by the supplier.

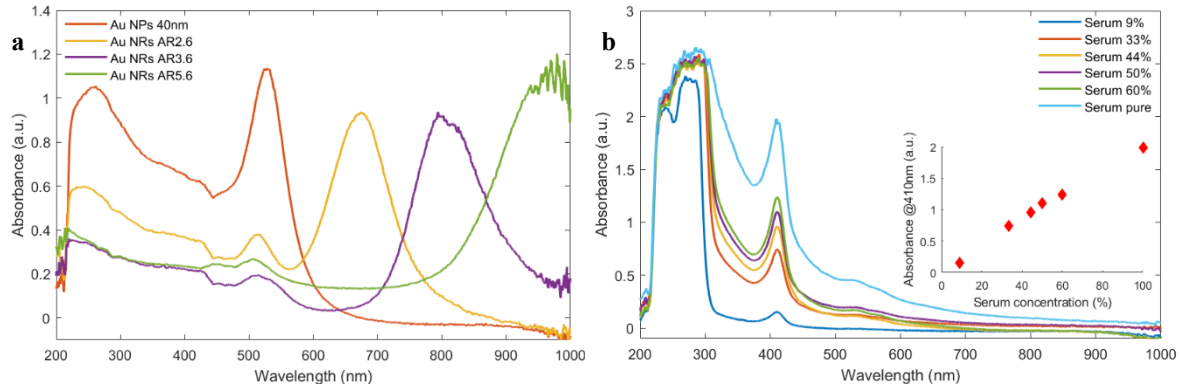


*Figure 1. (a) Absorbance spectra of gold NPs and NRs. (b) Absorbance spectra of filtered mouse serum for different concentrations; the inset shows the essentially linear increase of the absorbance peak at 410 nm.*

Mouse serum was also studied by absorbance spectroscopy, as shown in Fig.1(b). From the spectra, the absorbance peak in the ultraviolet range, a characteristic of proteins, is clearly visible, together with a peak at 410 nm. We do believe that this peak is related to enzymatic reactions, or to other components of mouse serum, and indeed it varies linearly with the concentration, as shown in the inset in Fig.1(b). For the following discussion we will mention proteins as the interactive components of mouse serum, although its composition is more differentiated.

DLS measurements were performed firstly on Au NPs and NRs suspensions, as shown in Fig.2(a). For Au NPs 40 nm, only the peak related to translational diffusion is present, and it has an average value of $R_H$ = 27.6 nm. It is well known that the hydrodynamic radius of particles in liquids is larger than the one of dried particles [12]; this can be explained by the formation of a corona around the particles, due to the interactions with the molecules within the liquid. The size distribution of Au NRs shows always two peaks (Fig.2(a)). One is related to translational diffusion, corresponding to a hydrodynamic radius of around 19 nm, and another is correlated to rotational motion,

corresponding to 2 nm radius. This is in agreement with earlier observations by DLS on non-spherical particles [7]. In spite of similar detections of rotational modes, to our knowledge it is still hard to identify the nanorods dimensions, i.e. diameter and length, by a standard DLS experiment. However, our data allow us to make some considerations about their shape. In fact, we observe that the standard deviation of the translational peak increases by increasing the aspect ratio; i.e. longer nanorods result in a broader distribution of the hydrodynamic radius, which can be related to translational motion (Fig.2(a)).

Samples of mouse serum at different concentrations were also analysed by DLS, the results are shown in Fig.2(b). The sample with 9% dilution was not enough concentrated for reliable DLS data, so we don't take it into account for the further analysis. A common feature of the DLS measurements on mouse serum is the presence of three different hydrodynamic radii in the size distribution:

- a small one, between 2 nm and 5 nm, which we associate to the rotational motion of proteins, and its scattered intensity can be an indication of the length of particles [7];
- a medium one, between 10 nm and 20 nm, is related to the translational motion, so it is the value conventionally measured by a DLS acquisition;
- a large one, above 100 nm corresponds to the hydrodynamic radius of agglomerates present in the sample, where the rotation and translation contributions are not distinguished anymore [6].

We note that from the concentration of 60% the position of the translational peak starts to increase, which may indicate increasing protein-protein interaction. Moreover, in the case of non-diluted serum (100% concentration) the hydrodynamic radius of agglomerates is larger. Even if the values are still inside the error bar, this trend is confirmed by the value of the diffusion coefficient, which is reduced by almost 50% (from $7.8 \cdot 10^{-12}$ $\pm 0.5 \cdot 10^{-12}$ $m^2/s$ for 33% concentration to $4.0 \cdot 10^{-12}$ $\pm 0.4 \cdot 10^{-12}$ $m^2/s$ for 100%). For these reasons, we have chosen a concentration of 33% for the following measurements. At this dilution, the hydrodynamic radius related to the translational peak is relatively small, providing the possibility to appreciate the interaction with added particles, such as NPs and NRs.

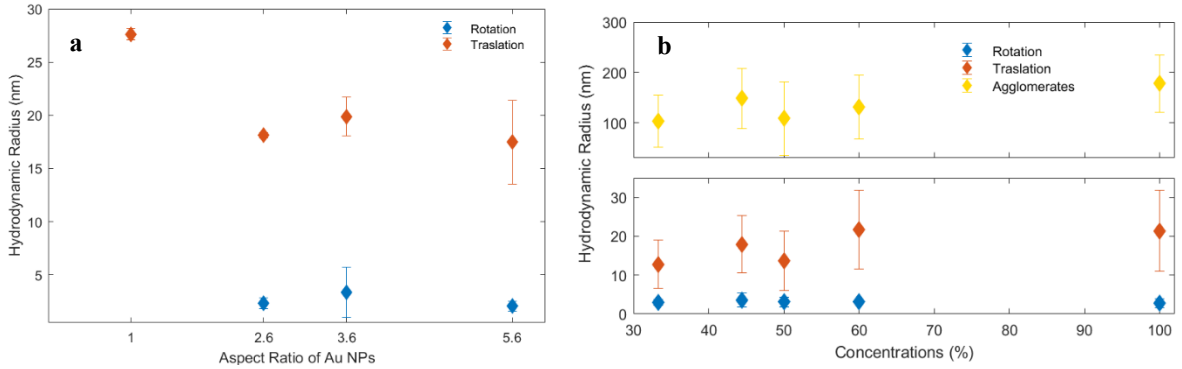


*Figure 2. (a) Hydrodynamic radius of Au NPs and NRs as a function of their aspect ratio. (b) Hydrodynamic radius of filtered mouse serum diluted at different concentrations.*

### 3.2 Interaction of Au NPs and mouse serum

Results of the size distribution analysis from DLS spectra of filtered mouse serum diluted at 33% and mixed with 7% of Au NPs and NRs are shown in Fig.3(a). The position labelled by "Serum 33% pure" corresponds to the bare sample. In the presence of Au NPs 40 nm (aspect ratio 1) and Au NRs AR2.6 the size distributions don't present significant changes, suggesting that there is not a remarkable NP-protein interaction. To the contrary, Au NRs AR3.6 presents an increase of the translation peak by almost 10 nm, and no agglomerates peak is present. A hypothesis that could explain this phenomenon is that the NRs-proteins interaction has as a consequence the formation of chains, and all the proteins get involved. Moreover, the big standard deviation of this peak indicates

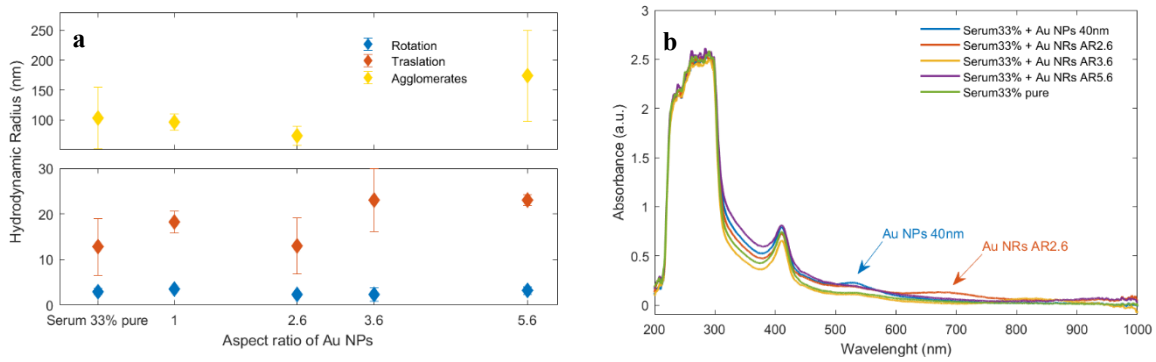


*Figure 3. Hydrodynamic radius (a) and absorbance spectra (b) of mouse serum diluted at 33% mixed with Au NPs and NRs.*

that the length of these chains has a broad dispersion. Au NRs AR5.6 shows another interesting behaviour: the agglomerates peak has a value significantly larger than the others samples, with a large standard deviation, while the translational peak has a value comparable to Au NRs AR3.6 but with a smaller standard deviation (Fig.3(a)). These observations suggest that in this case proteins and NRs are interacting in such a way to form spherical conglomerations with large hydrodynamic radii. The Au NPs and NRs studied in this paper have the same surface stabilization, (citrate), therefor we suppose that its stability changes according to the aspect ratio of the particles, in line with previous observations of the interaction of monodisperse proteins with Au NRs [7,13].

The interactions between proteins and NRs could be further investigated, for example, by Atomic Force Microscopy (AFM), in order to elucidate the shape of agglomerates and to determine the different behaviour related to the NRs' aspect ratio.

To conclude the optical analysis of our samples, we performed absorbance spectroscopy on mouse serum with added Au NPs and NRs, as shown in Fig.3 (b). The position and the intensity of the absorbance peaks stays constant, and the peaks characteristic of Au SPR bands are slightly visible only for Au 40 nm and for Au NRs 2.6, as indicated by the arrows in the figure. We can conclude that, from an optical point of view, the addition of Au NPs and NRs to mouse serum samples doesn't bring any substantial changes to the UV-Vis-NIR absorbance spectra.

## 4. CONCLUSIONS

In this study we have investigated the interaction of mouse serum with Au NPs and NRs by DLS. We have shown that with this technique we can distinguish three different peaks in the hydrodynamic radius distribution, each one related to a different type of motion (i.e. rotation, translation, agglomerates). Furthermore, we have found that the NRs-proteins interaction depends on the aspect ratio. In particular, particles with aspect ratio of 1 and 2.6 don't influence the proteins size distribution, while particles with aspect ratios of 3.6 and 5.6 stimulate the formation of agglomerates of different shape. A similar behaviour has been reported for the study of various monodisperse proteins [7,13]. In our study, instead, we show that it is possible to evaluate the interaction of NPs and NRs even in a more complex biological fluid (mouse serum) thanks to the advantages of DLS.

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