1 Adsorption of proteins on TiO₂ particles influences their 2 aggregation and cell penetration

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13 **Abstract**

14 TiO₂ nanoparticles known as E171 are one controversial food additive due to its potential toxicity.
15 In this work, the main hypothesis is that the proteins adsorbed on the TiO₂ nanoparticles prevent
16 their aggregation and favor the cell penetration. To do so, the TiO₂ nanoparticles were coated
17 with gelatin and β-lactoglobulin (two proteins commonly found in food) to reach interfacial
18 concentrations about 0.25 mg/mg and 0.32 mg/mg, respectively. The measurement of NP size
19 showed that the protein coating improve the colloidal stability of TiO₂ nanoparticles. The FTIR
20 analysis suggests that the β-lactoglobulin structure is modified after adsorption. Then, the
21 penetration of TiO₂ penetration inside human intestinal epithelial cells was shown and quantify
22 by using confocal Raman microscopy. Finally, the promoting role of the protein coating on the cell
23 penetration was demonstrated for both the gelatin and β-lactoglobulin.

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1. Introduction

Nanoparticles (NP) are commonly used in food as texturing and flavor agents (McCracken et al., 2016). One widely used additive is the food grade TiO_2 also identified as E171, which is found in various food products (including candies and chocolate bars) as well as toothpaste. In 2012, a simulation of TiO_2 consumption emphasizes that children below 10 years old are the most exposed individuals (Weir et al., 2012). TiO_2 is also used for packaging and, in that case, food can be contaminated by the packing (Lin et al., 2014). The question about the innocuousness of TiO_2 nanoparticles (TiO_2 NP) is not new since in 2011, Skocaj et al. addressed the question: "Titanium dioxide in our everyday life; is it safe?" (Skocaj et al., 2011). Closer than 10 years after, the impact of food grade TiO_2 on health is not totally elucidated and there is a need for interdisciplinary approaches to understand organ/NP interaction (Chaudhry et al., 2008).

Numerous effects of TiO_2 have been reported on different organs such as liver, brain, intestines or spleen (Jovanović, 2015). The TiO_2 NPs are known as inducers of inflammation in several organs such as kidney (Gui et al., 2011). In addition, genotoxicity has been observed for numerous structures of TiO_2 NPs (including anatase and rutile) with different exposure modes (ingestion, inhalation...) (Shi et al., 2013). The TiO_2 NPs induce oxidative damages to DNA. The E171 toxicity on intestinal cells is moderated but sufficient to suggest a role in tumor growth in the colon of mice (Dorier et al., 2017; Proquin et al., 2018). Moreover, E171 is suspected to worsen existing intestinal disease (Urrutia-Ortega et al., 2016). Indeed, the sedimentation of TiO_2 could induce a loss of intestinal microvilli (Faust et al., 2014) and a slight deregulation of the fatty acids profiles in the intestine. However, the TiO_2 NPs do not seem to impact the bacteria gas production (Dudefoi et al., 2017). Finally, in cells, TiO_2 NPs were found to disrupt the structure of lysosomes and to damage the mitochondria (Zhang et al., 2018) and could have synergy action with food molecules such as glucose (Wang et al., 2013).

The relevant properties which explain NP toxicity are their solubility, shape, surface charge, size distribution and structure (McCracken et al., 2016). Cell penetration was reported after dermal exposure and in hairless skin, TiO₂ NPs were found in epidermis cells (Wu et al., 2009). Basically, TiO₂ NPs can penetrate in cells as shown for MCF-7 and TERT cells using confocal Raman spectroscopy (Salehi et al., 2014). Recently, debate around the use of TiO₂ in food was revived. In 2017, Bettini et al. reported that after 100 days, the TiO₂ nanoparticles initiate preneoplastic lesions and promote colon inflammation. This suggested a potential role of TiO₂ NPs in autoimmune disease and colorectal cancers (Bettini et al., 2017). Following this publication, the French Agency for Food, Environmental and Occupational Health and Safety has recommended the limitation the TiO₂ as food additive as emergency measure regarding the lack of knowledge about the real toxicity (Anses, 2019).

As the other nanoparticles, TiO₂ tends to aggregate in solution. This is enhanced by the presence of salt in the media (Allouni et al., 2009). The ability of proteins to prevent nanoparticle aggregation is well known (Lepoitevin et al., 2015). For noble metals, such property is modulated to design colorimetric sensors (Sabela et al., 2017). The coating of TiO₂ NPs by human serum albumin, bovine serum albumin or fetal bovine serum prevents the aggregation process (Allouni et al., 2009; Yusoff et al., 2018). In their review, McCracken et al. hypothesized that NP coating by protein could be an important factor of their toxicity (McCracken et al., 2016).

Protein adsorption on material is a complex and fascinating phenomena whose mechanism is not fully understood after several decades of investigation. However, the structural classification of proteins could be a way to predict their behavior on a flat surface (Coglitore et al., 2019). Numerous parameters affect protein adsorption on nanoparticles such as pH, concentration, temperature, salt concentration (Bhakta et al., 2015) and additional molecules such as polyphenol (Coglitore et al., 2018). Hard proteins such as lysozyme keep their structure after adsorption on

solid interface (Balme et al., 2013). Although considered as soft protein, BSA does not unfold on food grade E171 (Kim & Doudrick, 2019). The adsorption of blood proteins on anatase TiO₂ NPs affects the agglomerate size and surface charge. Additionally, this altered the electrostatic binding affinity with fibroblasts (Allouni et al., 2015). Because proteins can strongly interact with TiO₂ NPs, we addressed the question about the consequences of such interaction. Typically, under salted media, NP aggregation reduces the ability of the NP to penetrate inside the cell. Food proteins, by preventing this aggregation process, could therefore enhance the penetration of TiO₂ NPs in the cell. In order to verify this hypothesis, we selected two proteins *i.e.* β -lactoglobulin and gelatin. β -lactoglobulin is mainly composed of β -sheets which provide a high internal energy. In presence of silica or hydrophobic surface, the conformational changes of β -lactoglobulin are small (Wahlgren & Arnebrant, 1990). When β -lactoglobulin is adsorbed on clay, only weak structural modification occur (Assifaoui et al., 2014). The second protein is gelatin, which is widely used in candies mixed with E171. Gelatin interacts with TiO₂ NPs and such interaction has been used to pattern TiO₂ porous microspheres (Liu et al., 2015). However, the main application of gelatin TiO₂ composite is food packaging (He et al., 2016).

Mostly, intracellular imaging methods, such as electron microscopy, cryoelectron microscopy, and immunofluorescence microscopy, due to the fixation, freezing and use of dyes or biomarkers are destructive and harmful to the cells. As Raman spectroscopy does not need chemical fixation, markers, or genetic modifications, it is considered as a non-invasive imaging method (Klein et al., 2012; Salehi et al., 2013). Raman spectroscopy is based on inelastic scattering of photons from the incident wavelength. The monochromatic light interacting with the sample, produces a Raman spectrum, composing different bands related to the vibrational frequencies of different functional groups. Subsequently every molecule has an exclusive fingerprint or Raman spectrum. The high spatial resolution and possibility of imaging in aqueous environment make Raman

microscopy an ideal tool for life and fixed imaging of a single cell (Salehi et al., 2014). The adequate data analysis according to the vibrational spectra of different cellular organelles, anticancer drugs or nanoparticles provides alternative to existing methods for cell imaging under normal physiological conditions (Gulka et al., 2020; Salehi et al., 2018). The unique spectral signature of nanoparticles enable tracing coated/ non-coated TiO₂ cellular penetration.

As previously mentioned, the toxicity of TiO₂ was demonstrated on different type cells in vitro as well as in-vivo. In these cases, numerous external parameters can influence the cell penetration of the nanoparticle including the protein adsorption. This work aims to verify for the first time the hypothesis of the role the protein coating on the TiO₂ nanoparticle cell penetration. Indeed, this question is motivated to elucidate the first step mechanism of the tiO₂ toxicity. To do so, gelatin and β -lactoglobulin adsorption on TiO₂ rutile was first investigated to determine their surface concentration and eventual structural modifications. Then, we evaluated the impact of each of the two proteins on TiO₂ NPs aggregation. Finally, the penetration of NPs coated or not with proteins was investigated by confocal Raman microscopy.

2. Materials and methods

a. Preparation of NPs

The titanium dioxide (TiO₂) powder (99% rutile) (SIGMA) was suspended in distilled water at a concentration of 1 mg/ml. The solution was then sonicated for 1 hour at 60°C (ref sonicator: ELMA Ultrasonic Cleaner S100H) to break up any interactions that may have formed between the different particles. Gelatin (L3908) and β -lactoglobulin (G6144) from Sigma were dissolved in distilled water at a concentration of 1 mg/ml and sonicated. Three solutions were prepared at a final concentration of 0.75 mg/ml of TiO₂ NPs: NPs alone, NPs with gelatin and NPs with β -lactoglobulin. In these solutions, the ratio was about 3 TiO₂ NPs to 1 protein. In order to promote

the adsorption of these proteins on NPs as effectively as possible, the solutions were placed on an agitator for one hour at room temperature and then stored at 4°C. A sonication of 5 minutes at room temperature for coated rated NPs and one hour at 60°C for unrated uncoated NPs was applied before each use.

b. Characterization of protein adsorption

The interfacial concentration of protein on TiO₂ NPs was obtained by the method of supernatant depletion previously reported (Lepoitevin et al., 2014). Briefly, after incubation, the mixtures containing TiO₂ NPs and proteins were centrifuged (15 min at 15000 rpm). Protein concentration in the supernatant was determined by UV-Vis absorption (Jasco) at 290 nm. The structural modifications of adsorbed protein were characterized by ATR-FTIR (Nexus) under D₂O. The measurement of NP size was performed by Dynamic Light Scattering (DLS) (Nanophox) using a laser light of 632 nm. For each condition or type of NPs, the measurement was carried out in triplicate. Several parameters such as medium (water, DMEM/F12 medium and RPMI medium) condition and time (from day 0 to day 5) were modified between different measurements. The data analysis was done using QuickFit software.

c. Cell culture

HT29 human colon adenocarcinoma cells were grown in DMEM/F12 medium (M1) or RPMI medium (M2) supplemented with 10% FCS, 100U/ml penicillin, 100 mg/ml streptomycin and 100 mg/ml sodium pyruvate. For Raman experiments, 3x10⁵ HT-29 cells were cultivated for 24 h onto polished and disinfected calcium fluoride CaF₂ (Crystran Ltd, Dorset, UK) substrates in 35 mm Petri dishes. After cell adherence to the substrate, the cells were incubated with dissolved nanoparticle solutions at 2 µg/mL concentration for one hour. The cells on the CaF₂ substrates were then fixed with 2% PFA (paraformaldehyde) after thorough rinsing with PBS. Cells were kept in PBS and transferred directly for Raman measurements. Before carrying out measurements of

TiO₂ on the cells, several reference spectra of the different NP solutions were carried out for 4 conditions, TiO₂ powder and the three solutions of TiO₂ coated or not by proteins (NP/NPG/NPL).

d. Analysis of cell penetration by Raman microscopy

Raman data acquisition

To collect the Raman spectra, Witec Confocal Raman Microscope System alpha 300R (Witec Inc., Ulm, Germany) was used. The excitation light in confocal Raman microscopy was generated via a frequency-doubled Nd:YAG lasers (New-port, Irvine, CA, USA) at a wavelength of 532 nm. A 60× NIKON water immersion objective with a numerical aperture of 1.0 and a working distance of 2.8 mm (Nikon, Tokyo, Japan) was used to focus the laser beam onto the cells. The laser power after the objective was 15 mW but finally, lower power was absorbed by cells in PBS. The scattered radiation goes through an edge filter to the electron multiplying charge coupled device camera EMCCD (DU 970N-BV353, Andor, Hartford, USA). The EMCCD chip size was 1600 × 200 pixels, the camera controller of a 16-bit A/D converter operated at 2.5 MHz. The acquisition time of a single spectrum was set to 0.5 sec. A zone of 150 × 150 pixels (spatial unit) per image was recorded, giving 22500 spectra for one image. Data acquisition and analysis was performed using Image Plus 2.08 Witec software. A spatial resolution of 300 nm and a depth resolution of 1µm were measured for the system. Considering the limit of Raman microscope (300 nm), the measurements of particle sizes on each image (processed and analyzed using Witec software) were done by zooming on particles and using line size measurement tool.

Raman data analysis

Two data analysis methods have been applied. The first method presented integrated Raman intensities in specific regions in particular C-H stretching mode. The lipid-protein distribution in the cells is shown by the integrated Raman intensities of C-H stretching mode (2800–3000cm⁻¹) using Image Plus software, Witec. Consequently, a map of the region regarding these integrated

intensities was provided. A false color image of CH Raman peak contains bright yellow hues for the highest intensities and dark orange hues for the lowest integrated intensities. K-mean cluster analysis (KMCA) as the second data analysis method separated data into k-mutually clusters. KMCA was done using the Witec Project Plus (Ulm, Germany) software.

e. Statistical analysis

All experiments were conducted independently at least three times. Results were expressed as the mean $\pm$ standard error of the mean (S.E.M). Statistical comparisons were performed with one-way ANOVA or t-test as indicated. A probability level (p value) of 0.05 was chosen for statistical significance.

3. Results and discussion

a. Protein adsorption on TiO₂ NPs

Prior to the investigation of the protein impact on colloidal stability and cell penetration, we characterized protein loading on TiO₂ NPs. Indeed, the protein/NP interaction is extremely dependent on the intrinsic properties of the material and on the media. Here, we have used water in order to prevent salt induced aggregation of TiO₂ NPs and thus to optimize the accessible surface of NPs for protein adsorption.

Figure 1: (A) Absorbance spectra of β -lactoglobulin (left) and gelatin (right) before (black line) and after (red line) contact with TiO₂ NPs at a concentration of 0.75 mg/ml for 1 hour. (B) FTIR spectra of β -lactoglobulin (left) and gelatin (right) in D₂O (black line) and loaded on TiO₂ NPs (blue or red line)

The interfacial concentration of protein was determined from the depletion of supernatant method. Figure 1A shows the absorbance spectra for β -lactoglobulin and gelatin before and after

adsorption on TiO₂ NPs. We can observe a large decrease of protein concentration in solution upon adsorption. The interfacial concentrations for β -lactoglobulin and gelatin were found about 0.25 mg/mg and 0.32 mg/mg of TiO₂ NPs, respectively.

We attempted to obtain information about the structural modifications of proteins induced by their adsorption on TiO₂ NPs. In Figure 1B are plotted the FTIR spectra recorded under D₂O. For the β -lactoglobulin, the band in 1640 cm⁻¹ was assigned to the amide I. In D₂O, the amides II gave two bands in 1560 cm⁻¹ and 1455 cm⁻¹ relative to N-H and N-D bonds, respectively. This emphasizes the accessibility to the solvent of the amide II moieties to exchange H by D. After loading on TiO₂ NPs, the 3 bands were still present. Unfortunately, the absorption of Ti-OD bond about 1590 made a deep structural analysis impossible. However, we observed an increase of the band in 1455 cm⁻¹ that could be assigned to the conversion of N-H to N-D. This suggested that the amides I bound with H were more exposed to the solvent after loading on TiO₂ NPs due to structural modifications.

For gelatin in D₂O, the 3 bands were also present but more difficult to distinguish because of the low order degree of the protein. However, we noticed that the two bands of amides I were large showing the existence of N-H and N-D bonds. After loading, the band about 1690 cm⁻¹ masked the other ones. Conversely to β -lactoglobulin, the adsorption of gelatin did not seem to promote the exposition of the amides II to the solution.

b. Colloidal stability of TiO₂ NPs with and without protein loading

As previously mentioned, the colloidal stability of TiO₂ NPs could strongly be affected by protein adsorption. Importantly, this is also a key factor for cell penetration. Thus, we then investigated by dynamic light scattering the TiO₂ NP size, in water and in two cell culture media.

Figure 2: Nanoparticle size by diffusion light scattering (A) Autocorrelation function of TiO₂ NPs without (black) and with protein (in red, β -lactoglobulin and in blue, gelatin). The sizes of TiO₂ NPs are obtained in water (B) and culture media (DMEM/F12 medium (M1) or RPMI medium (M2)) (C). (D) Size evolution of the small component as a function of time (From Day 0 to Day 5). TiO₂ NPs alone is noted NP, TiO₂ NPs with gelatin is noted NPG and TiO₂-NPs with β -lactoglobulin is noted NPL. Statistical significance is shown as p-value from one-way Anova test (panels B and C) or t-test in panel D (*: $p < 0.05$; *: $p < 0.001$).**

In Figure 2A, the autocorrelation functions for TiO₂ NPs in water, with and without proteins are reported. We observed a shift toward short times when TiO₂ NPs were coated with proteins. At first approximation, the autocorrelation functions were fitted with only one component to obtain an average size distribution. For uncoated TiO₂ NPs, the distribution was centered around 3.2 μ m

while after protein coating it was about 400 nm (Figure 2B). This is due to a rapid aggregation of TiO₂ NPs after sonication. Here, we can note that both gelatin and β -lactoglobulin prevented the fast aggregation phenomena of TiO₂ NPs.

In general, NP aggregation is favored by salt addition. Thus, similar analyses were performed using two different media used for cell culture (Figure 2C). This experiment was also motivated because the role of protein loading cannot be predicted since it is dependent on the protein and the eventual binding with small molecules, as shown for gold NP (Coglitore et al., 2018; Lepoitevin et al., 2015) or clay mineral (Trigueiro et al., 2018).

As expected, the size of raw TiO₂ NPs increased due to salt-induced aggregation. Conversely, coating with proteins appeared to prevent the aggregation process. These results agree with previous investigations showing that the extracellular polymeric substances from *Bacillus subtilis* improve the colloidal stability of TiO₂ NPs (Di Lin et al., 2017; Di Lin et al., 2016). Gelatin has a low internal energy structure and thus can optimize its conformation around the TiO₂ NPs. Conversely, β -lactoglobulin is mainly composed of β -sheet, has a high internal energy and is positively charged at pH 7.4. However, the FTIR revealed structural modifications, which probably allowed the interaction between the protein and TiO₂ NPs. This means that the electrostatic interaction between the TiO₂ NPs and β -lactoglobulin are not shielded by salt addition thus explaining the colloidal stability.

TiO₂ NPs penetrate inside cells only if their size are hundreds nanometer scale. The low accuracy of the autocorrelation fit with only one component suggested a polydispersity of the samples. To further analysis the size of TiO₂ NPs, we separated two populations from the autocorrelation curves. A first population with a diameter below 100 nm was assigned to non-aggregated particles. The second population micrometer scale corresponded to aggregates. The relative weight of each population showed that non-aggregated particles were the main one (upper than

99%), for the TiO₂ NPs coated with proteins. We then focused on this population more prone to penetrate inside the cells. The stability of these small TiO₂ NPs as a function of time was investigated for duration up to 5 days (Figure 2D). Without protein coating, TiO₂ NPs were prone to aggregate with time as monitored by the diameter variation as a function of the time. Typically, at D0 the mean size of the TiO₂-NP was about 100 nm and reached 480 nm after D5. This is not surprising since TiO₂ NPs are not stable in solution. With gelatin, the TiO₂ NPs size was stable until D1 around 91 nm. Then, the diameter variation increased to reach 250 % at D5. Finally, β -lactoglobulin offered the most effective protection against aggregation since the size of NPL particles remained constant during the 3-days.

At this stage, we demonstrated that protein coating prevented the TiO₂ NPs aggregation in water as well as in salt media. On the other hand, the colloidal stability was also improved during the time especially for the β -lactoglobulin.

c. Effect of proteins on cell penetration of TiO₂ NPs

To find out the role of protein, intracellular penetration of TiO₂ NPs was investigated using the human intestinal epithelial cell lines HT-29, cultured in DMEM-F12 medium. The characterization of TiO₂ NPs and their location were obtained using confocal Raman microscopy.

The Raman spectrum of TiO₂ powder showed three significant peaks at 275 cm⁻¹, 450 cm⁻¹ and 610 cm⁻¹ (Figure 3A). Coated/non-coated TiO₂ NPs in solution, showed the same peak position confirming the integrity of TiO₂ NPs. The low intensities of TiO₂ NPs coated with proteins (blue and red line for NPG and NPL, respectively) peaks were due to the coating, which avoids particles accumulation and lower Raman signal. In addition, powder signal was higher as compared to the NP in solution, as the number of particles under the laser spot was lower for the floating particles in solution.

Figure 3: Raman spectra of (A) TiO_2 powder, (B) TiO_2 -NPs (black line), TiO_2 -NPs with gelatin (blue line) and TiO_2 -NPs with β -lactoglobulin (red line), the inset is a zoom of $200\text{ cm}^{-1} - 700\text{ cm}^{-1}$ region. (C) Integration over Raman intensities of CH region, HT-29 cells incubated with TiO_2 NPGs and selected areas to focus on the TiO_2 presence inside the cell in pink color and outside in green. The image (D), (E) and (F) were obtained in presence of TiO_2 -NPs, TiO_2 -NPs with gelatin and TiO_2 -NPs with β -lactoglobulin respectively.

Figure 3(B-E) illustrates the image acquisition of cells in presence of TiO_2 NPs with and without protein coating. Raman images present the biomolecules and two clusters in pink (intracellular penetrated particles) and green (extracellular particles). The intensity of CH bonds is plotted in yellow hues have the maximum intensity (for proteins or lipids) and dark hues have zero intensity

of CH bonds (background and out of cells). After 1 hour incubation of HT-29 cells with raw nanoparticles, TiO₂ NPs aggregates were mainly present outside the cell or were in interaction with the cell membrane. Conversely, TiO₂ NPs coated with proteins were found inside the cells suggesting that protein coating favored cell penetration. We also noticed that TiO₂ NPs coated with proteins were more abundant as compared to the particles without coating.

Further analysis reported on Figure 4 shows the percentage of total penetration of coated/noncoated NPs. The quantification was obtained from a total number of 296 cells from all the Raman images that were analyzed. The average size of TiO₂ NPs inside the cells showed that the raw TiO₂ NPs were larger (1.4 μ m) than the coated TiO₂ NPs (1 μ m and 0.8 μ m for gelatin and β -lactoglobulin, respectively). The spatial resolution of Raman microscopy is 300 nm and thus a discrepancy between the DLS is not surprising. Indeed, the Raman microscopy provides information on several nanoparticles that can count one by one as soon as their size is larger than the resolution while the DLS provides a means measurement of the nanoparticle size that can be distorted in the case of heterogeneous samples. However, the tendency between confocal Raman microscopy results and DLS measurements were in good agreement.

We also quantified the percentage of NPs that penetrated inside the cells (Figure 4B and C). For the raw TiO₂ NPs, 35% of particles were located inside HT-29 cells. This ratio increased up to 42% and 55% when the TiO₂ NPs were coated with gelatin or β -lactoglobulin, respectively. Such enhancement of cell penetration induced by protein coating was significant and probably linked, at least in part, to the difference in size of the three types of NPs. We also evaluated the distribution of TiO₂ NPs in the cell population. Without proteins, the TiO₂ NPs penetrated only 28% of the cells. The coating with protein favored the dissemination of TiO₂ NPs since 48% and 70% of cells showed internalized TiO₂ NPs after coating with gelatin and β -lactoglobulin, respectively. The

311 difference between the two proteins can be assigned to their ability to prevent the TiO₂ NPs
312 aggregation.

313 As demonstrated, confocal Raman microscopy with high spatial resolution allowed us to analyze
314 and trace coated/non-coated TiO₂ nanoparticles. This label-free method using the spectral
315 fingerprint of nanoparticles permitted the monitoring of their intracellular penetration. The
316 results obviously present the significant difference between coated particles agglomeration and
317 penetration. Non-coated TiO₂ are more prone to aggregation and therefore their intracellular
318 penetration was lower. Conversely, protein adsorption prevented the TiO₂ NPs aggregation and
319 thus favored cell penetration.

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Figure 4: Characterization of TiO₂ NP penetration inside HT-29 cells (A) NP size, (B) ratio of intracellular TiO₂ NPs and (C) percentage of cells containing TiO₂ NPs. TiO₂ NPs alone is noted NP, TiO₂ NPs with gelatin is noted NPG, and TiO₂ NPs with β -lactoglobulin is noted NPL. Statistical significance is shown as p-value from one-way Anova test (*: $p < 0.05$; **: $p < 0.01$; *: $p < 0.001$).**

4. Conclusion

To sum up, we investigated the impact of protein adsorption on cell penetration of TiO₂ NPs. Our results showed that both β -lactoglobulin and gelatin were loaded on TiO₂ NPs. The FTIR suggested that β -lactoglobulin (but not gelatin) adsorption induced a structural modification. The salt-induced aggregation and the colloidal stability were improved by protein adsorption. The cell penetration investigated by confocal Raman microscopy revealed that the β -lactoglobulin favored more efficiently than gelatin TiO₂ NP cell penetration. Moreover, our results showed a correlation between the ability of proteins to prevent NP aggregation and to facilitate cell penetration.

Overall, this work proves that proteins present in food have a significant impact on TiO₂ NP penetration in human intestinal epithelial cells. This tends to reinforce the hypothesis that NP coating by proteins could be an important factor explaining their toxicity. Thus, in further investigations aiming to understand the toxicity of TiO₂ NP, the role of food proteins should be taken into account.

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