Published in final edited form as:

Nanoscale. 2017 November 23; 9(45): 17758–17769. doi:10.1039/c7nr02999c.

Probing the modulated formation of gold nanoparticle-beta lactoglobulin corona complexes and its applications

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Abstract

Understanding interactions between proteins and nanoparticles (NPs) along with the underlying structural and dynamic information is of utmost importance to exploit nanotechnology for biomedical applications. Upon adsorption onto the NP surface, proteins form a well-organized layer that dictates the identity of the NP-protein complex named corona and governs its biological pathways. Given the high biological relevance, in-depth molecular investigations and applications of NPs-protein corona complexes are still scarce, especially since different proteins form unique patterns of corona and hence identification of biomolecular motifs at the interface is critical. In this work, we provide molecular insights and structural characterizations of the bionano interface of a popular food-based protein, bovine beta-lactoglobulin (β -LG), with gold nanoparticles (AuNPs) and the formation of corona complexes by combined molecular simulations and complementary experiments. Two major binding sites in β -LG were identified to be driven by citrate-mediated electrostatic interactions, while the associated binding kinetics and conformational changes in secondary structures were also characterized. More importantly, the superior stability of the corona led us to further explore its biomedical applications with examples of smart-phone based point-of-care biosensing of *Escherichia coli* (*E. coli*) and computed tomography (CT) of gastrointestinal (GI) tract through oral administration to probe GI tolerance and functions. Considering the biocompatibility, edible nature and efficient excretion through

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defecation, AuNPs- β -LG corona complexes have shown promising perspectives in future *in vitro* and *in vivo* clinical settings.

Graphical Abstract

Keywords

gold nanoparticles; beta lactoglobulin; protein corona; discrete molecular dynamics; *E. COLI* biosensor; GI tract CT imaging

Introduction

By virtue of the high surface free energy of nanomaterials, protein molecules can bind to their surface to form an active biological coating, defined as protein corona. Protein-NPs corona complexes can be indeed considered as new nano-entities with the interfacial corona determining their identities and interacting with biological systems¹⁻². Recent studies have shown that protein corona rather than the NP core, is the effective unit governing cell-NP interactions and the major modulator of biological responses including immunogenicity and toxicity³. More noticeably, protein corona even decides biological fates of non-protein coated NPs and its formation undergoes a rapid process in less than one minute⁴⁻⁵. Distinct binding dynamics and structural changes of proteins can occur upon protein-NPs complexing, which are dominated by materials, surface, size and shape of NP cores as well as molecular weights and three dimensional structures of proteins⁶⁻⁷. Serum protein corona composition with different NPs has been studied in detail using proteomics⁴⁻⁵. However, the complexity of these coronae makes them unsuitable for further applications. Although model systems like transferrin-polystyrene-NPs and ubiquitin-AgNPs have been well-studied⁷⁻⁸, comprehensive investigations on intermolecular interactions and binding kinetics for other entirely different protein-NPs systems are still in great demand. For instance, serum protein corona could enhance MRI contrasts of negatively-charged superparamagnetic iron oxide NPs (SPIONs)⁹ and improve biocompatibility of NPs¹⁰. Pre-formed ¹²⁵I-radiolabeled protein corona on ⁵⁹Fe-SPION facilitated cellular uptakes and showed superior stability¹¹. It was also reported that bovine serum albumin (BSA) corona formation conferred high colloidal stability and prevented the aggregation and precipitation of citrate-stabilized AuNPs beyond physiological salt concentrations (150 mM NaCl)¹². Granting all these interesting features, practical applications of modulated protein-NPs corona complexes are

still far from widely explored, especially for those none serum proteins based systems. Bearing that in mind, we aim to design a biocompatible corona-NPs complex, probe their interactions, and explore their biomedical applications.

AuNPs are among the list of most attractive nanomaterials for biosensing, imaging and therapeutics, owing to their inertness, unique optical properties, biocompatibilities and tailored surface modifications¹³. In addition, compared to some other metal nanoparticles like Ag and Cu with high cytotoxicity, citrate-capped AuNPs and Au nanoshells have been explored in early clinical trials by systemic administration without major adverse effects^{14–15} along with many other trials still ongoing, while AuNPs-based VeriGene® system is commercially available for *in vitro* detection of bacteria in patient samples^{16–17}. This *in vitro* diagnostic system has been cleared by FDA, but there are no Au agents approved so far for *in vivo* applications, though metallic gold-decorated deserts are being sold for oral ingestion. Oral administration of certain sized AuNPs did not produce observable systemic toxicities¹⁸ and only a tiny amount of oral dose would be absorbed and retained in the body¹⁹. β -LG, the major whey protein component in cow's milk, is an 18.4 kDa protein with 162 residues involved in molecular transport and immune modulation^{20–21}. β -LG is a clinically critical antigen associated to milk allergy and plays an indispensable role in vaccination and oral tolerance induction^{22–23}. Nonetheless, oral protein delivery has major difficulties in achieving sufficient bioavailability and pharmacological efficacies influenced by acidic stomach environment, digestive enzymes in GI tract, high molecular weight and structural stabilities of proteins²⁴. On the other hand, complexation of proteins with NPs may provide an emerging viable solution to these issues²⁵. AuNPs- β -LG complexes could be a promising oral administered agent and avoid potential high uptake in reticuloendothelial system from systemic administration. CT is one of the most extensively-used radiological diagnostic imaging techniques in clinical settings to provide anatomical information. Compared to small molecule-based iodine, NPs have longer blood circulation half-life and target tissue retention time, allowing for prolonged imaging availability, reduced renal toxicity, minimized hypersensitive reactions and better contrast enhancements^{26–27}. Re-administration can be avoided in case of multiple examinations. Mass-energy absorption coefficient of Au is much higher than that of clinical iodinated contrast agents in the mammography and clinical CT windows, along with a larger K-edge absorption (Au 80.7 keV vs. I 33.2 keV)²⁸, making AuNPs an ideal substitute. CT scan of GI tract is usually a mandatory prerequisite for diagnosis of GI diseases. GI intolerance can also pose negative impacts on compliance with therapeutic regimens and affect bioavailability and thus monitoring GI functions in a non-terminal *in vivo* way is an unmet need.

In the present work, we studied interactions between β -LG and AuNPs by discrete molecular dynamics (DMD) simulations and complementary experiments and identified electrostatic interaction as the driving force to form corona complexes. DMD simulations, a flavor of molecular dynamics algorithm with high computational sampling efficiency, have been successfully applied to study the nano-bio interface featuring large system sizes and high molecular complexities^{29–30}. In DMD simulations, two major binding motifs driven by citrate-mediated electrostatic interactions were identified and the observed conformational changes of β -LG secondary structures were consistent with experimental characterizations.

The AuNP- β -LG also features high colloidal stability. With this modulated corona complex, we constructed a digital image analysis (DIA)-based optical biosensor for point-of-care detection of *E. coli* as a rapid screening alternative to the time-consuming colony counting and insensitive optical density methods. Besides the *in vitro* application, we exemplified a proof-of-concept *in vivo* imaging application of GI tract using CT modality to probe anatomical details and GI functions.

Experimental

Materials

High-purity bovine milk β -LG (BioPURE) composed of genetic variants A and B was obtained from Davisco Foods International Inc. (Eden Prairie, MN) with detailed compositions as shown in Table S1. Gold(III) chloride trihydrate ($\text{HAuCl}_4\text{K}_3\text{H}_2\text{O}$) was acquired from Sigma-Aldrich and all other chemicals in the study were all of analytical grade and used without further purifications. Ultrapure deionized Milli-Q water (18.2 M Ω) was used in the entire work.

Instrumentation

UV-vis spectra were recorded using Cary 50 Bio UV-vis spectrophotometer and zeta potentials were determined by Brookhaven 90Plus nanoparticle size analyzer with BI-Zeta module. Attenuated total reflectance-Fourier Transform infrared spectroscopy (ATR-FTIR) was carried out with PerkinElmer Spectrum 100. Fluorescence spectroscopy was measured using QuantaMaster Model C-60/2000 spectrofluorimeter at the excitation wavelength of 295 nm to minimize tyrosine fluorescence. Scanning electron microscopy and transmission electron microscopy were performed with Hitachi S-4800 and FEI Tecnai T-12 respectively. Olympus fluorescence microscope BX 53 coupled with mercury-vapor short arc lamp (X-cite series 120Q) as light source and LMPlanFL N 20x lens was used to record images of β -LG intrinsic fluorescence on Olympus DP72 cooled CCD camera with 330 and 420 nm excitation and emission filter sets at an exposure time of 60 s. A pH Meter (Accumet Research AR50) was used to monitor the pH adjustments by 1 M HCl or NaOH.

Synthesis of gold nanoparticles

All glassware were thoroughly cleaned by freshly prepared aqua regia ($\text{HCl}:\text{HNO}_3$ at molar ratio 3:1) and water to avoid potential undesired nucleation during synthesis and aggregation of gold colloids. 1.7 mL 0.1 M trisodium citrate was quickly added into a boiling solution of 50 mL 1 mM HAuCl_4 solution under stirring of 400 rpm. After the color changed from pale yellow to dark red within 10 min indicative of AuNPs formation, the solution was allowed to stand and be cooled at room temperature with continuous stirring for another 15 min. The resulting solution was filtered through a sterile 0.22 μm syringe filter and titrated to 50 mL before use. The molar concentration of AuNPs (C_{Au}) is calculated to be 12.1 nM based on Eq. (2) and (3), assuming spherical shapes and uniform fcc structure^{31–32}:

$$N = \frac{\pi \rho D^3}{6M} \quad (2)$$

$$C_{Au} = \frac{n}{N} \quad (3)$$

where N is the number of Au atoms per NP, ρ is density of fcc Au at $193 \text{ g}\cdot\text{cm}^{-3}$, M is Au atomic weight of $197 \text{ g}\cdot\text{mol}^{-1}$, D is the core diameter and n refers to the precursor molar concentration. All AuNPs concentrations in the study are given as gold atomic molar concentrations instead of AuNPs molar concentrations unless otherwise noted.

Preparation of AuNPs- β -LG corona complexes

Fresh β -LG solutions were prepared each time from powder before use by dissolving in water, passing through $0.22 \mu\text{m}$ filter and being shaken at 100 rpm at room temperature for 2 hr to achieve dissolution and partial hydration. AuNPs- β -LG corona conjugates were prepared by mixing AuNPs and β -LG and thermodynamically stabilized at room temperature with intermittent mild vortexing for 30 min. To maintain the potential conformational states and prevent damages and disruptions of the corona complexes from shear stresses, no further centrifugation and filtration were carried out unless otherwise noted. Concentration-dependent colloid stability was evaluated to decide the optimal capping concentration for corona formation.

Computational modeling

DMD is special type of molecular dynamic simulation algorithm with enhanced sampling efficiency, which has been extensively used to model protein folding, protein-ligand interactions as well as protein-nanoparticle interactions^{7, 33}. We adopt a united-atom representation of simulated molecules, where all heavy atoms and polar hydrogens are explicitly modelled. The calculated interatomic interactions include *van der Waals*, solvation, electrostatic interactions and hydrogen bond. The distance- and angular-dependent hydrogen bond interactions are modelled using a reaction-like algorithm³⁴. The solvation energy is estimated with the Lazaridis-Karplus implicit solvent model, EEF1³⁵. Screened electrostatic interactions are modelled by the Debye-Hückel approximation. A Debye length of 1 nm is used by assuming a water dielectric constant of 80 and a monovalent electrolyte concentration of 0.1 M. All DMD simulations are conducted at room temperature, $\sim 300 \text{ K}$. The Anderson's thermostat is used to for the constant temperature simulations³⁶. We adopt the recently developed Au molecular mechanics force field to model Au surface³⁷. More specifically, the AuNP is modeled by a (111) Au surface with 5 atom layers. The corresponding force field of the AuNP includes both physical and chemical absorptions, aromatic interactions as well as "image" charge interactions. The "image" charge interaction is modeled with polarizable dipoles, where a freely-rotatable dipole is attached to each metal atom. We simply attach a charged virtual atom ($-0.3e$) to each metal ($0.3e$) atom with a fixed bond length ($1.0 \text{ \AA}$). Except the electrostatic interaction, the virtual atoms do not have any other interaction. The initial structure coordinates of β -lactoglobulin (β -LG) is obtained from the RCSB Protein Data Bank³⁸ with the PDB ID 4y0p. The basic and acidic residues of for the β -LG are assigned charges corresponding to their titration states at physiological condition ($\text{pH}=7.4$) – i.e. Arg and Lys residues are assigned $+1e$ while Asp and Glu are

assigned 1e, while His was neutral. The citrate molecule is negatively charged ($-3e$) with three carboxyl groups. The dimension of Au surface is set as $\sim 8 \times 8$ nm and initially fully covered with citrates. β -LG was assigned near the AuNP surface with fifteen random distances/orientations. We perform fifteen independent simulations each of 62.5 ns and an accumulative 0.9375 μ s of total DMD simulation time in order to acquire sufficient sampling. β -LG alone is also simulated at same amount of time as control for further structural comparison. Counter ions (Na^+) are added accordingly to maintain the net charge of each system zero and account for possible counter-ion condensation³⁹. Energy minimizations are performed for the system prior to the productive simulations.

Preparation of *E. coli* and DIA-based detection

E. coli Dh5 α cultures were grown in Luria-Bertani (LB) broth at 37°C for 24 h. The relationship between optical density at 600 nm (OD_{600}) and increasing population of bacteria was monitored and verified by conventional LB agar plate approach. A sample of 1×10^{11} CFU/mL of *E. coli* at exponential growth phase was picked up and diluted to desired concentrations. The concentrations of *E. coli* before performing the detection were also verified by plate culture. A portable Iphone 5S camera was used to take digital images of solutions of AuNPs- β -LG corona complexes in a transparent vial in the presence and absence of *E. coli*. DIA was performed using standard RGB model. Equal amount of pixels for each sample were analyzed for RGB profiles and R/G ratios were calculated using Image J.

In vivo CT imaging

BALB/cJ white mice at 8–12 weeks were purchased from the Jackson Laboratory and maintained under an environment complied with NIH guidelines for care and use of laboratory animals. AuNPs- β -LG corona complexes were exchanged into pH 7.4 1x PBS, washed extensively (five times) to remove unbound citrates and finally concentrated, using 3 kDa cutoff filters (Amicon® Ultra) before gavage. Mice weighed at 21 ± 2 g were on a fasting diet for 12 h before being orally administered 800 μ L of AuNPs- β -LG corona complexes (5.0 mM Au) in PBS using gavage needles and imaged using CT modality of NanoSPECT/CT (Mediso Medical Imaging, MA) at the voltage of 65 kVp and exposure time of 1500 ms. CT slices were reconstructed using InVivoScope software and quantitatively calculated using Image J.

Results and discussion

The citrate-stabilized AuNPs showed a strong absorption in the visible range peaked at 519 nm, a well-known feature of localized surface plasmon resonance (LSPR) band for 14 nm AuNPs due to collective surface electron oscillations with specific incident light (Fig. S1). After mixing β -LG with citrate-stabilized AuNPs, the absorption peak of AuNPs shifted bathochromically by about 7 nm to 526 nm as induced by the change in surrounding dielectric environments⁴⁰, accompanied by a sharp peak around 278 nm in the UV region attributed to the aromatic tryptophan and tyrosine residues of β -LG. Thus we note that the spectra of AuNPs- β -LG corona complexes are distinctly different in the SPR band position

with the mathematical spectral overlay of individual AuNPs and β -LG, suggesting the formation of β -LG protein corona.

DMD simulations were performed to study the binding of β -LG with citrate-coated AuNP. Given the relatively larger size of AuNP compared to β -LG (i.e., the radius of gyration $R_g \sim 1.5$ nm), the AuNP was modeled by a (111) Au surface covered with citrates. Control simulations of β -LG alone indicated that the protein remained at its stable native state at room temperature as elucidated by time-dependent root-mean-square deviation (RMSD; Fig. S2). Fifteen independent binding simulations were performed by initially positioning β -LG ~ 35 Å away from Au surface with varied orientations. In all cases, β -LG eventually bound to the gold surface (Fig. S3). Examination of a typical trajectory for the binding process of β -LG and AuNPs shows that as the distance between the center of β -LG and surface of AuNPs (d_{CM}) decreases and the number of atomic contacts (N_C) increases, the RMSD of β -LG increases from its native state upon protein-NP binding (e.g., near 15 ns in Fig. S4), indicating a binding event at the interface along with re-orientation and conformational changes of β -LG. Before the binding event occurs, d_{CM} has a marked large initial fluctuation of 30–40 Å, followed by a rapid decrease with relatively small RMSD implying protein re-orientation. It is also noteworthy that after binding, β -LG could further partially unfold with increase in RMSD and slightly spread onto the surface of AuNPs with a smaller d_{CM} (after 55 ns simulations as highlighted in Fig. S4). Both experimental and computational data above support the complex formation between β -LG and AuNP.

The absorbance of AuNPs at 580 nm is a sensitive indicator of aggregations^{41–42}. Therefore, the difference or ratio between the LSPR absorbance at 520 nm and 580 nm is used to probe the colloid stability at a fixed concentration of AuNPs at pH 4 (Fig. S5). AuNPs are not highly stable at this pH due to neutralization of surface charge^{43–44}. Proteins are optimally adsorbed onto metal surface at pH close to isoelectric point (pI) and deviations from pI result in decreased adsorption⁴⁵. At low β -LG concentrations, the insufficiently-capped AuNPs tend to be unstable with electrostatic interactions between negatively charged AuNPs and positively charged acidic residues (i.e., lysine and arginine) of β -LG. The threshold concentration required to encapsulate AuNPs and maintain a stable colloidal solution was determined to be 0.5–1 mgK_ML⁻¹, as seen by the largest absorption difference, indicating highest stability. Complete corona formation by β -LG ligands are helpful in keeping stability and preventing further aggregations. It then reached a plateau and started to slightly decrease, possibly due to protein self-aggregations at high concentrations⁴⁶ coordinated by inter-protein hydrogen bond which in turn cause minor aggregations of AuNPs. In addition, as shown in Fig. S6, low β -LG concentrations did not induce significant red shifts in peaks of AuNPs in the visible region while showing intrinsic concentration-dependent absorptions in the UV region. Unlike citrate-stabilized AuNPs which are unstable at low pH and high salt conditions^{43, 47}, β -LG–AuNPs corona complexes showed enhanced stability at low and high pH and high salt concentrations without observable aggregations (Fig. S7). In fact, in addition to inhibition of aggregation induced by neutralization, the binding affinity of proteins to citrate-capped AuNPs was reported to increase in the presence of salt¹².

A representative TEM image of AuNPs in spherical shape showed good monodispersity of a size of 13.9 ± 1.0 nm (Fig. 1A). After forming β -LG corona, transparent protein shells of

approximately 2.5 ± 0.3 nm entrapped around the AuNP cores were observed. The AuNPs- β -LG corona complexes are stabilized and embedded in β -LG matrix, as seen by the mushy areas and obscured smear around AuNPs in SEM images (Fig. S8). The size histogram of corona complexes with a Au metal core and protein shell suggests a larger size of 18.9 ± 3.7 nm than AuNPs, with a similar width of size distribution except a weak tail of large sizes (Fig. 1A), consistent with the bathochromically-shifted absorption peak observed in Fig. S1. Since the native structure of β -LG is approximately 3.8 nm in diameter ($D \sim 2Rg/0.6^{1/2}$ assuming a simple spherical structure) which is larger than its thickness, the adsorbed corona β -LG should have undergone certain conformational changes as observed in simulations (e.g., the high decrease of absorbed protein highlighted by the red box in Fig. S4). The small fraction of AuNPs- β -LG corona with large sizes might correspond to small AuNP aggregates formed during the incubation with β -LG where the exchange with citrates took place.

From the atomistic DMD simulations, an ensemble of AuNPs- β -LG complex was constructed to uncover the binding structures of β -LG on the NP surface and the conformational changes of proteins upon NP binding. The binding probability of each β -LG residue with AuNPs at molecular levels (Fig. S9) featured several distinct peaks where a portion of amino acids have significantly higher binding frequencies than the others which barely interact. Most of these residues with high NP binding frequencies (>0.45) are positively charged and cluster together on the protein surface, corresponding to the surface area with high electrostatic potential (Fig. 1B). Since the NP-binding surface of protein is uneven, two distinct binding modes were observed where the corresponding binding interfaces were adjacent to each other (Fig. 1C and 1D). The binding preference can be attributed to the citrate coating on the NP surface as negatively charged citrates on the NP surface drove the binding of β -LG with its positively charged surface. While the dynamic displacement of citrates was observed (Fig. S10), the overall binding interface of β -LG kept the same due to its strong binding with NP. Electrostatic interaction was therefore determined to be the major driving force in forming complexes and surface-capped citrate has a great influence in the binding behaviors, mechanistically different from the case of hydrophobic interaction-ruled AuNPs-BSA corona formation¹². Changes in secondary structures of β -LG were computed for the last 25 ns where the protein was bound in all simulations. Our simulations indicated that β -LG manifested a decrease in β -sheet and α -helix and an increase in random coil and β -turn levels (Fig. S11). Residues with decreased β -sheet and α -helix propensities correspond to these in contact with the NP (Fig. S9 and S12), suggesting that the loss of ordered secondary structures are induced by NP binding.

The isoelectric point (pI) of AuNPs- β -LG corona complexes under optimal conditions was determined, at pH where zeta potential is zero, to be 4.88 (Fig. 2A), which is almost identical to that of β -LG (≈ 4.8)^{48–49}, confirming formation of complete corona. FTIR spectra of corona complexes are shown with AuNPs and β -LG as reference spectra (Fig. S13A). Peaks of AuNPs at 1398 and 1599 cm^{-1} (red dotted lines) correspond to stretching vibrations of carboxylate groups of citrate⁵⁰, but similar peaks also exist in β -LG (Fig. S13B) due to protein side-chain carboxylates. Asymmetric in-plane bending of CH_3 from side chains and imidazole ring of tryptophan induce peaks at 1352 and 1448 cm^{-1} respectively⁵¹, whereas strong alkene fingerprint bands at 2875, 2935 and 2960 cm^{-1} are

attributed to CH₃ symmetric, CH₂ and CH₃ asymmetric stretching vibrations⁵². Peaks of amide I band around 1634 cm⁻¹ mainly originate from C=O stretching, closely related to backbone conformation, while peaks of amide II band around 1545 cm⁻¹ are attributed to in-plane N-H bending and C-N stretching vibrations⁵¹. Peaks at 3280 cm⁻¹ of amide A and 3086 cm⁻¹ of amide B bands result from Fermi resonance between the first overtone of amide II and N-H stretching vibration. Due to the broadening effect in microenvironment, amide I envelope with its shape and peak position as a sensitive indicator of protein secondary structure, is Fourier self-deconvoluted (FSD) by Gaussian fitting (Fig. S14). Native β-LG results in a secondary structural composition of β-sheet, random coil, α-helix and β-turn at 53.1%, 4.9%, 15.3% and 26.8% respectively, well correlated to previous reported values⁵³. Upon formation of AuNPs-β-LG corona complexes, fractions of ordered secondary structures such as β-sheet and α-helix are decreased and altered into unordered structures, as observed in the increase of random coil, while β-turn fraction almost remains identical (Fig. 2B). The experimentally observed protein secondary structure changes were consistent with the predicted trends of secondary structure changes from all-atom DMD simulations (Fig. S11). Compared to experimental observations, the smaller decreases of β-sheet and α-helix contents in simulations suggests that β-LG may undergo further partial unfolding on the NP surface, which takes place at longer time scales (Fig. S4). It is noteworthy to point out that these secondary structural changes are different from ubiquitin-AgNPs and amyloid β-AuNPs systems⁶⁻⁷.

Steady-state intrinsic tryptophan (Trp) fluorescence intensity of β-LG decreased with increasing concentrations of AuNPs which behave as a quencher upon forming complexes (Fig. 2C). Meanwhile, the shape and position of emission peaks largely stayed unchanged with increasing AuNPs concentrations, a typical feature of water-soluble quenchers⁵⁴. This strongly suggests minor variations in the Trp microenvironment except for those quenched Trp residues sufficiently close to Au core. As a matter of fact, in both binding modes we identified, one Trp residue is always close to the binding interface (Trp⁶¹ and Trp¹⁹ for major and secondary binding modes respectively) while the other stays further (Fig. 1D). Stern–Volmer plot reflects an apparent upward exponential curving, attributed to a simultaneous dynamic and static quenching that can be fitted by sphere-of-action mechanism using Eq 1:

$$\frac{F_0}{F} = (1 + K_D[Q])e^{V[Q]} \quad (1)$$

where F and F₀ are fluorescence intensities in the presence and absence of AuNP quenchers, Q refers to quencher concentration, K_D is dynamic quenching constant, V represents the static quenching-related spherical volume around fluorophores where quenchers are active. A small K_D of 0.16 M⁻¹ is derived, indicating residues in β-LG are relatively inaccessible to solution-phase AuNPs and the predominant quenching is static, by forming complexes⁵⁵⁻⁵⁶. Similar observations were also reported in other nanocomplexes such as carbon nanotubes⁵⁷⁻⁵⁸ and Au clusters^{56, 59}. Such quenching is also verified by fluorescence microscopy (Fig. S15).

OD₆₀₀ is the most common absorbance-based approach for rapid estimation of bacteria in liquid, since the gold standard counting colony-forming units (CFU) in plate culture is labor-intensive and time-consuming. However, OD₆₀₀ is extremely insensitive and can only detect *E. coli* at a limit around 2×10^7 CFU·mL⁻¹ (Fig. S16), similar to previous finding for *Bacillus cereus*⁶⁰. Given the high LSPR sensitivity of AuNPs depending on surface-bound materials, we rationally constructed an optical biosensor utilizing the binding of positively-charged AuNPs-β-LG corona complexes with negatively-charged *E. coli*. Positively charged residues such as Lys⁴⁷ and Lys¹³⁸ have been shown to be the most exposed and reactive groups in β-LG⁶¹. Although the majority of acidic residues comprise the binding interface of AuNPs-β-LG corona complexes with Lys¹³⁸ near the binding surface, we found that several Lys residues in the corona including Lys⁴⁷, Lys⁶⁰, Lys⁶⁹ and Lys⁷⁰ remain solvent exposed (Fig. 1D). The zeta potential of AuNPs-β-LG corona complexes was 10.5 ± 1.9 mV at pH 4 along and *E. coli* cells were at -39.1 ± 2.6 mV with anionic bacterial membrane, consistent with previous investigations^{62–63}. Such electrostatic aggregation-based detection mechanisms were similarly applied on Au nanorods for photoacoustic detection and cysteine-modified AuNPs for optical detection of *E. coli*⁶⁴. While media did not cause any aggregations, a strong plasmon band red-shift of corona complexes were observed in the presence of *E. coli* (Fig. 3A). *E. coli* suspension alone does not have any characteristic peaks in this region^{64–65}. Surprisingly, the plasmon band first bathochromically and then hypsochromically shifted with increasing bacterial concentrations in the range we investigated (Fig. 3B and Fig. S17), demonstrating a threshold for interactions of corona complexes and *E. coli*. At low concentrations, all cells were saturated and bound with corona complexes and excessive complexes are not further bound to each other, due to their high colloid stability. In contrast, at high concentrations, corona complexes were insufficient to saturate all *E. coli* cells, leading to a lower degree of aggregation. This result was also confirmed by TEM where citrate-AuNPs with strong negative charges barely interact with *E. coli*, and AuNPs-β-LG corona complexes bind by neutralizing surface charge of *E. coli* through electrostatic interactions and perturbing bacterial envelope upon binding saturation (Fig. 3C). Noticeably, some dispersed unbound corona complexes were observed beyond binding saturation proving our hypothesis. Such a binding mechanism with a saturation threshold is widely adopted in a number of antimicrobial peptides^{63, 66}. Indeed, glycosylation and multiple isolated peptides from bovine β-LG were identified with antibacterial properties^{67–68} and the partial unfolding and conformational changes through binding with AuNPs could help expose these active motifs. These strongly suggested some other interaction mechanisms may exist, besides electrostatic interactions (Fig. S18), such as hydrophobic interactions between protein and bacterial membrane lipids^{69–70}, given the fact that bovine β-LG is extensively used as carriers for lipids and other hydrophobic drugs as a core lipocalin^{71–73}. Dative bond between Au and conducting electrons of S and N atoms from proteins could also be involved⁴⁵.

Although such detection can be practically achieved by naked human eyes, the spectral sensitivities are inferior to camera sensors. As an inexpensive point-of-care substitute for conventional spectrophotometric approaches to be used under resource-limited settings, DIA with smart phones elucidates specific red-green-blue (RGB) color profiles of AuNPs-β-LG corona complexes. GB values decrease drastically with only minor variations in R value, as

AuNPs concentration increases (Fig. S19). In contrary, RGB values only have minor variations with changing concentrations of β -LG (Fig. S20). We found that an upward exponential correlation ($R/G \text{ ratio} = 0.23 * \exp(C_{Au}/0.11) + 0.80$, $R^2 = 0.994$) can be established between R/G ratios and AuNPs concentrations, but R/G ratios of a fixed AuNPs concentration almost remain constant, independent of β -LG concentrations (Fig. 3D). In the current work, we found R/G ratio to be the most sensitive and stable indicator with a high correlation coefficient and thus can serve as a potential responsive indicator for aggregation under the same AuNPs concentration, different from detections with AuNPs using G/(R+G+B) for heparin⁷⁴, R/B for DNA⁷⁵, G-B for Pb²⁺⁷⁶ and R for quinidine⁷⁷. AuNPs- β -LG corona complexes in the absence of *E. coli* and AuNPs in the presence of *E. coli* both displayed a R/G value similar or lower than 1.4 (Fig. 3E) which is the cutoff of 0.1 mM AuNPs- β -LG (Fig. 3D). Even in the presence of relatively low concentrations of *E. coli* which only induce mild aggregations hardly differentiated by naked eye, a significantly higher R/G value can be obtained by DIA, validating the feasibility and potential using AuNPs- β -LG corona complexes as *E. coli* biosensors.

Given the edible nature of both Au and β -LG, we further explored the use of AuNPs- β -LG corona complexes for contrast-enhanced CT imaging to probe GI functions and tracked its fate in GI tract. Taking advantages of superior electron density ($19.32 \text{ g}\cdot\text{cm}^{-3}$) and high atomic number (79) of Au which are major determinants of attenuation coefficients⁷⁸, *in vitro* phantom imaging of AuNPs- β -LG corona complexes showed increasing CT contrasts at higher concentrations, much stronger than PBS control (Fig. 4A). *In vivo* non-invasive whole-body imaging revealed enhanced contrasts in the upper digestive system including stomach and first several sections of small intestine such as duodenum and jejunum, 30 min following oral administrations of AuNPs- β -LG corona complexes (Fig. 4B). The corona complexes went further into ileum and cecum at 1 h and advanced to colons of large intestines from 2 h on, reaching a high-contrast point of anatomical information at 5 h (Fig. S21). Arrangements of intestinal loops were clearly outlined. 8 h post administration, small intestines were almost completely emptied whereas large intestines were filled. At 12 h, distinct sigmoid colon and rectum were visualized and its signal last till 48 h but became weaker over time due to defecation of corona complexes through anus. Gastric emptying kinetics of AuNPs- β -LG corona complexes exhibited a unique pattern of gradually increased CT signals in earlier time points and excreted after 24 h (Fig. 4C). This is different from optical gelling agents where decreasing signals were observed unless pectin was administered^{79–80}, while the corona complexes manifested a much longer retention time of 12 h in stomach in comparison. We also noticed that the CT-visualized volume of stomach decreased significantly after 8 h, in spite of increasing signals. Similar phenomenon was seen with silica-coated Bi₂S₃ nanorods⁸¹. Although our corona complexes showed appreciable stability at pH 2, digestive enzymes in stomach are likely to decompose the corona shell and change crystalline structures of AuNPs and different crystal faces lead to different X-ray attenuations⁸². Notably, stomach signals completely disappeared 24 h after administration. Furthermore, *ex vivo* CT imaging of excreted feces displayed normal fecal morphology with enhanced CT contrasts after administration (Fig. 4D), indicating AuNPs- β -LG corona complexes did not induce GI dysfunctions and can be efficiently expelled

through defecation pathway. Therefore, long-term body retention of the complexes can be largely avoided to induce minimized systemic toxicities.

Conclusion

In summary, we presented an in-depth investigation on interactions between β -LG and AuNPs by both simulations and experiments. β -LG can effectively replace stabilizing surface ligands, specifically bind to AuNPs through electrostatic interactions and form a protein corona around AuNPs. Binding kinetics were explored and two major surface binding sites of β -LG were identified, along with destabilization of ordered secondary structures such as α -helices and β -sheets. The AuNPs- β -LG corona complexes demonstrated superior stabilities at different pH and salt conditions. With the corona complexes, we constructed a point-of-care colorimetric biosensor for rapid detection of *E. coli*, simply relying on a smart phone camera. Due to the biocompatible nature and high X-ray attenuation, *in vivo* CT imaging of GI tract by oral administration was explored to evaluate GI functions and observe the fate of corona complexes. *Ex vivo* imaging confirmed the corona complexes could be efficiently excreted from the body by defecation within 24 h and thus pose minimized toxicity. Our study strongly suggested the potential of protein corona complexes, not limited to β -LG, can implement versatile biomedical applications such as biosensing and edible contrast agents for contrast-enhanced CT imaging of GI functions and diseases as well as surveillance of therapeutic GI treatments in clinical settings. Other applications including but not limited to mammography, fluoroscopy and CT-guided biopsy are yet to be further explored.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors are thankful to Hjalmar D. and Janet W. Bruhn Fellowship, Louis and Elsa Thomsen Wisconsin Distinguished Graduate Fellowship at UW-Madison and the Recruitment Program of Global Youth Experts of China provided to J.Y. The work was also supported by the NIGMS award R35GM119691 and NSF award CBET-1553945 to F.D.. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH and NSF.

References

1. Monopoli MP, Aberg C, Salvati A, Dawson KA. Biomolecular coronas provide the biological identity of nanosized materials. *Nat Nano*. 2012; 7(12):779–786.
2. Walczyk D, Bombelli FB, Monopoli MP, Lynch I, Dawson KA. What the Cell “Sees” in Bionanoscience. *J Am Chem Soc*. 2010; 132(16):5761–5768. [PubMed: 20356039]
3. Lee YK, Choi EJ, Webster TJ, Kim SH, Khang D. Effect of the protein corona on nanoparticles for modulating cytotoxicity and immunotoxicity. *Int J Nanomedicine*. 2015; 10:97–113. [PubMed: 25565807]
4. Schottler S, Becker G, Winzen S, Steinbach T, Mohr K, Landfester K, Mailander V, Wurm FR. Protein adsorption is required for stealth effect of poly(ethylene glycol)- and poly(phosphoester)-coated nanocarriers. *Nat Nano*. 2016; 11(4):372–377.
5. Tenzer S, Docter D, Kuharev J, Musyanovych A, Fetz V, Hecht R, Schlenk F, Fischer D, Kiouptsi K, Reinhardt C, Landfester K, Schild H, Maskos M, Knauer SK, Stauber RH. Rapid formation of

plasma protein corona critically affects nanoparticle pathophysiology. *Nat Nano*. 2013; 8(10):772–81.

6. Kim Y, Park JH, Lee H, Nam JM. How Do the Size, Charge and Shape of Nanoparticles Affect Amyloid β Aggregation on Brain Lipid Bilayer? *Sci Rep*. 2016; 6:19548. [PubMed: 26782664]
7. Ding F, Radic S, Chen R, Chen P, Geitner NK, Brown JM, Ke PC. Direct observation of a single nanoparticle-ubiquitin corona formation. *Nanoscale*. 2013; 5(19):9162–9169. [PubMed: 23921560]
8. Kelly PM, Aberg C, Polo E, O'Connell A, Cookman J, Fallon J, Krpeti željka; Dawson KA. Mapping protein binding sites on the biomolecular corona of nanoparticles. *Nat Nano*. 2015; 10(5): 472–479.
9. Amiri H, Bordonali L, Lascialfari A, Wan S, Monopoli MP, Lynch I, Laurent S, Mahmoudi M. Protein corona affects the relaxivity and MRI contrast efficiency of magnetic nanoparticles. *Nanoscale*. 2013; 5(18):8656–8665. [PubMed: 23896964]
10. Landgraf L, Christner C, Storck W, Schick I, Krumbein I, Dahring H, Haedicke K, Heinz-Herrmann K, Teichgraber U, Reichenbach JR, Tremel W, Tenzer S, Hilger I. A plasma protein corona enhances the biocompatibility of Au@Fe₃O₄ Janus particles. *Biomaterials*. 2015; 68:77–88. [PubMed: 26276693]
11. Bargheer D, Nielsen J, Gebel G, Heine M, Salmen SC, Stauber R, Weller H, Heeren J, Nielsen P. The fate of a designed protein corona on nanoparticles *in vitro* and *in vivo*. *Beilstein J Nanotechnol*. 2015; 6:36–46. [PubMed: 25671150]
12. Dominguez-Medina S, Blankenburg J, Olson J, Landes CF, Link S. Adsorption of a Protein Monolayer via Hydrophobic Interactions Prevents Nanoparticle Aggregation under Harsh Environmental Conditions. *ACS Sustain Chem Eng*. 2013; 1(7):833–842. [PubMed: 23914342]
13. Cheheltani R, Ezzibdeh RM, Chhour P, Pulaparthi K, Kim J, Jurcova M, Hsu JC, Blundell C, Litt HI, Ferrari VA, Allcock HR, Sehgal CM, Cormode DP. Tunable, biodegradable gold nanoparticles as contrast agents for computed tomography and photoacoustic imaging. *Biomaterials*. 2016; 102:87–97. [PubMed: 27322961]
14. Libutti SK, Paciotti GF, Byrnes AA, Alexander HR Jr, Gannon WE, Walker M, Seidel GD, Yuldasheva N, Tamarkin L. Phase I and pharmacokinetic studies of CYT-6091, a novel PEGylated colloidal gold-rhTNF nanomedicine. *Clin Cancer Res*. 2010; 16(24):6139–49. [PubMed: 20876255]
15. El-Sayed IH. Nanotechnology in Head and Neck Cancer: The Race Is On. *Curr Oncol Rep*. 2010; 12(2):121–128. [PubMed: 20425597]
16. Wojewoda CM, Sercia L, Navas M, Tuohy M, Wilson D, Hall GS, Procop GW, Richter SS. Evaluation of the Verigene Gram-positive blood culture nucleic acid test for rapid detection of bacteria and resistance determinants. *J Clin Microbiol*. 2013; 51(7):2072–6. [PubMed: 23596240]
17. Beal SG, Ciurca J, Smith G, John J, Lee F, Doern CD, Gander RM. Evaluation of the nanosphere verigene gram-positive blood culture assay with the VersaTREK blood culture system and assessment of possible impact on selected patients. *J Clin Microbiol*. 2013; 51(12):3988–92. [PubMed: 24048531]
18. Jo M-R, Bae S-H, Go M-R, Kim H-J, Hwang Y-G, Choi S-J. Toxicity and Biokinetics of Colloidal Gold Nanoparticles. *Nanomaterials*. 2015; 5(2):835. [PubMed: 28347039]
19. Chen H, Dorrigan A, Saad S, Hare DJ, Cortie MB, Valenzuela SM. In vivo study of spherical gold nanoparticles: inflammatory effects and distribution in mice. *PLoS One*. 2013; 8(2):e58208. [PubMed: 23469154]
20. Roth-Walter F, Pacios LF, Gomez-Casado C, Hofstetter G, Roth GA, Singer J, Diaz-Perales A, Jensen-Jarolim E. The major cow milk allergen Bos d 5 manipulates T-helper cells depending on its load with siderophore-bound iron. *PLoS One*. 2014; 9(8):e104803. [PubMed: 25117976]
21. Tai CS, Chen YY, Chen WL. Beta-Lactoglobulin Influences Human Immunity and Promotes Cell Proliferation. *BioMed Res Int*. 2016; 2016:12.
22. Pecquet S, Leo E, Fritsche R, Pfeifer A, Couvreur P, Fattal E. Oral tolerance elicited in mice by β -lactoglobulin entrapped in biodegradable microspheres. *Vaccine*. 2000; 18(13):1196–1202. [PubMed: 10649620]

23. Rytönen J, Karttunen TJ, Karttunen R, Valkonen KH, Björkstén B, Kokkonen J. BCG vaccine modulates intestinal and systemic response to β -lactoglobulin. *Pediatr Allergy Immunol*. 2004; 15(5):408–414. [PubMed: 15482515]
24. Carino GP, Mathiowitz E. Oral insulin delivery. *Adv Drug Deliv Rev*. 1999; 35(2–3):249–257. [PubMed: 10837700]
25. Wang XQ, Zhang Q. pH-sensitive polymeric nanoparticles to improve oral bioavailability of peptide/protein drugs and poorly water-soluble drugs. *Eur J Pharm Biopharm*. 2012; 82(2):219–29. [PubMed: 22885229]
26. Kim D, Park S, Lee JH, Jeong YY, Jon S. Antibiofouling Polymer-Coated Gold Nanoparticles as a Contrast Agent for in Vivo X-ray Computed Tomography Imaging. *J Am Chem Soc*. 2007; 129(24):7661–7665. [PubMed: 17530850]
27. Ahn S, Jung S, Lee S. Gold Nanoparticle Contrast Agents in Advanced X-ray Imaging Technologies. *Molecules*. 2013; 18(5):5858. [PubMed: 23685939]
28. Jackson PA, Rahman WNWA, Wong CJ, Ackerly T, Geso M. Potential dependent superiority of gold nanoparticles in comparison to iodinated contrast agents. *Eur J Radiol*. 2010; 75(1):104–109. [PubMed: 19406594]
29. Wang B, Blin T, Kakinen A, Ge X, Pilkington EH, Quinn JF, Whittaker MR, Davis TP, Ke PC, Ding F. Brushed polyethylene glycol and phosphorylcholine for grafting nanoparticles against protein binding. *Polym Chem*. 2016; 7(45):6875–6879. [PubMed: 28348639]
30. Wang B, Seabrook SA, Nedumpully-Govindan P, Chen P, Yin H, Waddington L, Epa VC, Winkler DA, Kirby JK, Ding F, Ke PC. Thermostability and reversibility of silver nanoparticle-protein binding. *Phys Chem Chem Phys*. 2015; 17(3):1728–1739. [PubMed: 25461673]
31. Liu X, Atwater M, Wang J, Huo Q. Extinction coefficient of gold nanoparticles with different sizes and different capping ligands. *Colloids Surf B Biointerfaces*. 2007; 58(1):3–7. [PubMed: 16997536]
32. Li P, Li D, Zhang L, Li G, Wang E. Cationic lipid bilayer coated gold nanoparticles-mediated transfection of mammalian cells. *Biomaterials*. 2008; 29(26):3617–3624. [PubMed: 18571230]
33. Radic S, Nedumpully-Govindan P, Chen R, Salonen E, Brown JM, Ke PC, Ding F. Effect of fullerene surface chemistry on nanoparticle binding-induced protein misfolding. *Nanoscale*. 2014; 6(14):8340–9. [PubMed: 24934397]
34. Ding F, Borreguero JM, Buldyrey SV, Stanley HE, Dokholyan NV. Mechanism for the alpha-helix to beta-hairpin transition. *Proteins*. 2003; 53(2):220–8. [PubMed: 14517973]
35. Lazaridis T, Karplus M. Effective energy functions for protein structure prediction. *Curr Opin Struct Biol*. 2000; 10(2):139–45. [PubMed: 10753811]
36. Andersen HC. Molecular dynamics simulations at constant pressure and/or temperature. *J Chem Phys*. 1980; 72(4):2384–2393.
37. Hughes ZE, Wright LB, Walsh TR. Biomolecular Adsorption at Aqueous Silver Interfaces: First-Principles Calculations, Polarizable Force-Field Simulations, and Comparisons with Gold. *Langmuir*. 2013; 29(43):13217–13229. [PubMed: 24079907]
38. Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, Shindyalov IN, Bourne PE. The Protein Data Bank. *Nucleic Acids Res*. 2000; 28(1):235–242. [PubMed: 10592235]
39. Manning GS. Limiting Laws and Counterion Condensation in Polyelectrolyte Solutions I. Colligative Properties. *J Chem Phys*. 1969; 51(3):924–933.
40. Iosin M, Toderas F, Baldeck PL, Astilean S. Study of protein–gold nanoparticle conjugates by fluorescence and surface-enhanced Raman scattering. *J Mol Struct*. 2009; 924–926:196–200.
41. Ho YT, Poinard B, Yeo ELL, Kah JCY. An instantaneous colorimetric protein assay based on spontaneous formation of a protein corona on gold nanoparticles. *Analyst*. 2015; 140(4):1026–1036. [PubMed: 25501998]
42. Ambrosi A, Airo F, Merkoci A. Enhanced Gold Nanoparticle Based ELISA for a Breast Cancer Biomarker. *Anal Chem*. 2010; 82(3):1151–1156. [PubMed: 20043655]
43. Basu S, Ghosh SK, Kundu S, Panigrahi S, Praharaj S, Pande S, Jana S, Pal T. Biomolecule induced nanoparticle aggregation: Effect of particle size on interparticle coupling. *J Colloid Interface Sci*. 2007; 313(2):724–734. [PubMed: 17540397]

44. Wang A, Ng HP, Xu Y, Li Y, Zheng Y, Yu J, Han F, Peng F, Fu L. Gold Nanoparticles: Synthesis, Stability Test, and Application for the Rice Growth. *J Nanomater.* 2014; 2014:6.
45. Thobhani S, Attree S, Boyd R, Kumarswami N, Noble J, Szymanski M, Porter RA. Bioconjugation and characterisation of gold colloid-labelled proteins. *J Immunol Methods.* 2010; 356(1–2):60–69. [PubMed: 20188107]
46. Treuheit MJ, Kosky AA, Brems DN. Inverse Relationship of Protein Concentration and Aggregation. *Pharm Res.* 2002; 19(4):511–516. [PubMed: 12033388]
47. Sakura T, Takahashi T, Kataoka K, Nagasaki Y. One-pot preparation of mono-dispersed and physiologically stabilized gold colloid. *Colloid and Polym Sci.* 2005; 284(1):97–101.
48. Harnsilawat T, Pongsawatmanit R, McClements DJ. Characterization of β -lactoglobulin– sodium alginate interactions in aqueous solutions: A calorimetry, light scattering, electrophoretic mobility and solubility study. *Food Hydrocoll.* 2006; 20(5):577–585.
49. Haug IJ, Skar HM, Vegarud GE, Langsrud T, Draget KI. Electrostatic effects on β -lactoglobulin transitions during heat denaturation as studied by differential scanning calorimetry. *Food Hydrocoll.* 2009; 23(8):2287–2293.
50. Kamelipour N, Mohsenifar A, Tabatabaei M, Rahmani-Cherati T, Khoshnevisan K, Allameh A, Milani MM, Najavand S, Etemadikia B. Fluorometric determination of paraoxon in human serum using a gold nanoparticle-immobilized organophosphorus hydrolase and coumarin 1 as a competitive inhibitor. *Microchim Acta.* 2014; 181(1):239–248.
51. Barth A. Infrared spectroscopy of proteins. *Biochim Biophys Acta-Bioenergetics.* 2007; 1767(9): 1073–1101.
52. Zhao X, Yang X, Shi Y, Chen G, Li X. Protein and lipid characterization of wheat roots plasma membrane damaged by Fe and H₂O₂ using ATR-FTIR method. *J Biophys Chem.* 2013; 4(1):28–35.
53. Ngarize S, Herman H, Adams A, Howell N. Comparison of Changes in the Secondary Structure of Unheated, Heated, and High-Pressure-Treated β -Lactoglobulin and Ovalbumin Proteins Using Fourier Transform Raman Spectroscopy and Self-Deconvolution. *J Agric Food Chem.* 2004; 52(21):6470–6477. [PubMed: 15479009]
54. Lehrer S. Solute perturbation of protein fluorescence. Quenching of the tryptophyl fluorescence of model compounds and of lysozyme by iodide ion. *Biochem.* 1971; 10(17):3254–3263. [PubMed: 5119250]
55. Schlamadinger DE, Kats DI, Kim JE. Quenching of Tryptophan Fluorescence in Unfolded Cytochrome c: A Biophysics Experiment for Physical Chemistry Students. *J Chem Educ.* 2010; 87(9):961–964. [PubMed: 25593364]
56. Yu P, Wen X, Toh Y-R, Lee Y-C, Tang J. Optical properties of gold particle-cluster coresatellite nanoassemblies. *RSC Adv.* 2013; 3(42):19609–19616.
57. Ahmad A, Kurkina T, Kern K, Balasubramanian K. Applications of the static quenching of rhodamine B by carbon nanotubes. *Chemphyschem.* 2009; 10(13):2251–5. [PubMed: 19630055]
58. Goutam PJ, Singh DK, Iyer PK. Photoluminescence Quenching of Poly(3-hexylthiophene) by Carbon Nanotubes. *J Phys Chem C.* 2012; 116(14):8196–8201.
59. Wu TH, Hsu YY, Lin SY. A redox-switchable Au₈-cluster sensor. *Small.* 2012; 8(13):2099–105. [PubMed: 22511503]
60. Biesta-Peters EG, Reij MW, Joosten H, Gorris LG, Zwietering MH. Comparison of two optical-density-based methods and a plate count method for estimation of growth parameters of *Bacillus cereus*. *Appl Environ Microbiol.* 2010; 76(5):1399–405. [PubMed: 20081006]
61. Del Moral-Ramirez E, Dominguez-Ramirez L, Cruz-Guerrero AE, Rodriguez-Serrano GM, Garcia-Garibay M, Gomez-Ruiz L, Jimenez-Guzman J. Role of Lysine ϵ -Amino Groups of β -Lactoglobulin on Its Activating Effect of *Kluyveromyces lactis* β -Galactosidase. *J Agric Food Chem.* 2008; 56(14):5859–5863. [PubMed: 18582081]
62. Kłodzka E, Szumski M, Dziubakiewicz E, Hryniewicz K, Skwarek E, Janusz W, Buszewski B. Effect of zeta potential value on bacterial behavior during electrophoretic separation. *Electrophoresis.* 2010; 31(9):1590–1596. [PubMed: 20422634]
63. Alves CS, Melo MN, Franquelim HG, Ferre R, Planas M, Feliu L, Bardaji E, Kowalczyk W, Andreu D, Santos NC, Fernandes MX, Castanho MARB. *Escherichia coli* Cell Surface

- Perturbation and Disruption Induced by Antimicrobial Peptides BP100 and pepR. *J Biol Chem.* 2010; 285(36):27536–27544. [PubMed: 20566635]
64. Liopo A, Derry PJ, Ermolinsky B, Su R, Conjesteau A, Ermilov S, Zubarev ER, Oraevsky A. Controlled bacteria-gold nanorod interactions for enhancement of optoacoustic contrast. *Proc SPIE.* 2014:894368.
 65. Raj V, Vijayan AN, Joseph K. Cysteine capped gold nanoparticles for naked eye detection of *E. coli* bacteria in UTI patients. *Sens Biosensing Res.* 2015; 5:33–36.
 66. Kiefer J, Ebel N, Schlucker E, Leipertz A. Characterization of *Escherichia coli* suspensions using UV/Vis/NIR absorption spectroscopy. *Anal Methods.* 2010; 2(2):123–128.
 67. Melo MN, Ferre R, Castanho MA. Antimicrobial peptides: linking partition, activity and high membrane-bound concentrations. *Nat Rev Microbiol.* 2009; 7(3):245–50. [PubMed: 19219054]
 68. Pellegrini A, Dettling C, Thomas U, Hunziker P. Isolation and characterization of four bactericidal domains in the bovine beta-lactoglobulin. *Biochim Biophys Acta.* 2001; 1526(2):131–40. [PubMed: 11325534]
 69. Moises Laparra J, Corzo-Martinez M, Villamiel M, Javier Moreno F, Sanz Y. Maillard-type glycoconjugates from dairy proteins inhibit adhesion of *Escherichia coli* to mucin. *Food Chem.* 2011; 129(4):1435–1443.
 70. Melo MN, Ferre R, Castanho MARB. Antimicrobial peptides: linking partition, activity and high membrane-bound concentrations. *Nat Rev Micro.* 2009; 7(3):245–250.
 71. Huang HW. Action of Antimicrobial Peptides: Two-State Model. *Biochem.* 2000; 39(29):8347–8352. [PubMed: 10913240]
 72. Ragona L, Zetta L, Fogolari F, Molinari H, Perez DM, Puyol P, Kruif KD, Lohr F, Ruterjans H. Bovine β -lactoglobulin: Interaction studies with palmitic acid. *Protein Sci.* 2000; 9(7):1347–1356. [PubMed: 10933500]
 73. Perez OE, David-Birman T, Kesselman E, Levi-Tal S, Lesmes U. Milk protein–vitamin interactions: Formation of beta-lactoglobulin/folic acid nano-complexes and their impact on in vitro gastro-duodenal proteolysis. *Food Hydrocol.* 2014; 38:40–47.
 74. Wang Q, Allen JC, Swaisgood HE. Binding of Vitamin D and Cholesterol to β -Lactoglobulin1. *J Dairy Sci.* 1997; 80(6):1054–1059. [PubMed: 9201574]
 75. Hemmateenejad B, Dorostkar S, Shakerizadeh-Shirazi F, Shamsipur M. pH-independent optical sensing of heparin based on ionic liquid-capped gold nanoparticles. *Analyst.* 2013; 138(17):4830–7. [PubMed: 23826612]
 76. Tsai TT, Shen SW, Cheng CM, Chen CF. Paper-based tuberculosis diagnostic devices with colorimetric gold nanoparticles. *Sci Technol Adv Mater.* 2013; 14(4):044404. [PubMed: 27877589]
 77. de L, de Moraes MC, Carvalho JC, Sant'Anna C, Eugenio M, Gasparotto LHS, Lima KMG. A low-cost microcontrolled photometer with one color recognition sensor for selective detection of Pb²⁺ using gold nanoparticles. *Anal Methods.* 2015; 7(18):7917–7922.
 78. Zou B, Liu Y, Yan X, Huang C. Gold nanoparticles based digital color analysis for quinidine detection. *Chin Sci Bull.* 2013; 58(17):2027–2031.
 79. Popovtzer R, Agrawal A, Kotov NA, Popovtzer A, Balter J, Carey TE, Kopelman R. Targeted Gold Nanoparticles Enable Molecular CT Imaging of Cancer. *Nano Lett.* 2008; 8(12):4593–4596. [PubMed: 19367807]
 80. Gremlich HU, Martinez V, Kneuer R, Kinzy W, Weber E, Pfannkuche HJ, Rudin M. Noninvasive assessment of gastric emptying by near-infrared fluorescence reflectance imaging in mice: pharmacological validation with tegaserod, cisapride, and clonidine. *Mol Imaging.* 2004; 3(4):303–11. [PubMed: 15802046]
 81. Yamaoka I, Kikuchi T, Endo N, Ebisu G. Fluorescence imaging in vivo visualizes delayed gastric emptying of liquid enteral nutrition containing pectin. *BMC Gastroenterol.* 2014; 14:168. [PubMed: 25263497]
 82. Zheng X, Shi J, Bu Y, Tian G, Zhang X, Yin W, Gao B, Yang Z, Hu Z, Liu X, Yan L, Gu Z, Zhao Y. Silica-coated bismuth sulfide nanorods as multimodal contrast agents for a non-invasive visualization of the gastrointestinal tract. *Nanoscale.* 2015; 7(29):12581–12591. [PubMed: 26145146]

83. Xi D, Dong S, Meng X, Lu Q, Meng L, Ye J. Gold nanoparticles as computerized tomography (CT) contrast agents. *RSC Adv.* 2012; 2(33):12515–12524.

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Figure 1. Interaction mechanisms between β -LG and AuNPs

(A) TEM images of (a) AuNPs and (b) AuNPs- β -LG corona complexes. TEM focus is set on the protein shell for better visualization. Inset shows the magnification of a single AuNPs- β -LG corona complex. (c) Histograms of particle size distributions for AuNPs and AuNPs- β -LG corona complexes. A schematic structure of AuNPs- β -LG corona complexes is shown as inset of (b). (B) The computationally derived surface mapping of AuNPs-binding frequency (upper) and electrostatic potential in β -LG (lower), revealing multiple binding motifs. (C) Two major binding sites identified in β -LG structure. Blue: rare binding sites; yellow and red: frequent binding sites (>0.45); yellow: charged residues. (D) Binding modes of β -LG to AuNPs. Green spheres: Trp¹⁹ and Trp⁶¹; gray sticks: solvent exposed Lys⁴⁷, Lys⁶⁰, Lys⁶⁹ and Lys⁷⁰.

Figure 2. Characterization of AuNPs- β -LG corona complexes

(A) Zeta potential of AuNPs- β -LG corona complexes at different pH. Isoelectric point is shown by the arrow. (B) Experimentally derived compositions of protein secondary structures for β -LG and AuNPs- β -LG corona complexes by analysis of mid-IR spectra. (C) Fluorescence emission spectra of 1.0 mg/mL β -LG with 0, 0.05, 0.10, 0.15 and 0.20 mM AuNPs from (a) to (e). The arrow indicates increasing concentrations of AuNPs with decreased fluorescence intensities and inset shows the Stern-Volmer plot of β -LG with concentration-dependent quenching by AuNPs.

Figure 3. Point-of-care detection of *E. coli* using AuNPs-β-LG corona complexes

(A) UV-vis spectra of AuNPs-β-LG corona complexes with or without 4.5×10^7 *E. coli* in culture media at pH 4. (B) *E. coli* concentration-dependent peak wavelength function of AuNPs-β-LG corona complexes. As the bacterial concentration increases, peak red shifts were observed followed by blue shifts. (C) TEM images of (a) *E. coli*, (b) *E. coli*+ AuNPs and (c) *E. coli*+ AuNPs-β-LG corona complexes. (D) Red/green ratios for RGB profiles of AuNPs-β-LG corona complexes from colorimetric results. (●) 0.1 mM Au with different β-LG concentrations of 0, 0.1, 0.2, 0.5, 1.0 and 2.0 mg/mL. (●) 1.0 mg/mL β-LG with 0, 0.05, 0.10, 0.15 and 0.2 mM Au. Projections onto each plane are shown for better visualizations. (E) Image-based sensitive colorimetric detection of *E. coli* in water by R/G ratios using AuNPs-β-LG corona complexes. The controls of citrate-capped AuNPs with and without *E. coli* are included. The dotted line shows the detection cutoff threshold.

Figure 4. *In vivo* CT imaging of GI tract using AuNPs- β -LG corona complexes

(A) MIP, transverse and coronal CT phantom images and quantitative calculations of PBS control and AuNPs- β -LG corona complexes (2.5 mM and 5.0 mM Au). (B) *In vivo* CT imaging of GI tract in BALB/cJ white mice at different time points post oral administration of AuNPs- β -LG corona complexes. Stomach regions are highlighted by green circles and do not have observable signals either at baseline levels after fasting or after 24 h post administration. (C) Gastric emptying kinetics evaluated by non-invasive whole-body CT imaging with equal ROI area. (D) MIP, sagittal and coronal CT images of excreted feces collected before or 24 h after administration.