Supplementary Materials for

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

Jiang Yang^{1,2,#,*}, Bo Wang^{3,#}, Youngsang You², Woo-jin Chang⁴, Ke Tang⁵, Yi-Cheng Wang², Wenzhao Zhang⁶, Feng Ding^{3*}, Sundaram Gunasekaran^{2*}

¹State Key Laboratory of Oncology in South China, Collaborative Innovation Center for Cancer Medicine, Sun Yat-sen University Cancer Center, Guangzhou 510060, China

²Department of Biological Systems Engineering, University of Wisconsin-Madison, 460 Henry Mall, Madison, WI 53706, USA

³Department of Physics and Astronomy, Clemson University, 118 Kinard Laboratory, Clemson, SC 29634, USA

⁴Department of Mechanical Engineering, University of Wisconsin-Milwaukee, 3200 North Cramer Street, Milwaukee, WI 53211, USA

⁵Department of Bioengineering, University of Illinois at Chicago, 851 South Morgan Street, Chicago, IL 60607, USA

⁶Department of Engineering Professional Development, University of Wisconsin-Madison, 432 North Lake Street, Madison, WI 53706, USA

*These authors contributed equally to this work

*Corresponding authors at jyang44@uwalumni.com (J.Y.), fding@clemson.edu (F.D.) and guna@wisc.edu (S.G.)

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Composition	Value
Protein (% total dry mass)	97.4
β-LG (% total proteins)	95.9
Variant A (% β-LG)	55.6
Variant B (% β-LG)	44.4
Fat (%)	0.1
Ash (%)	2.4
Moisture (%)	5.8
pH of 10% β-LG at 20°C	7.3
Lactose (%)	0.0
E. coli	Negative
Salmonella sp.	Negative
<i>Listeria</i> sp.	Negative

Table S1: Detailed physiochemical compositions of β -LG used in the study. Data were provided by Davisco Foods International Inc.



Figure S1: UV-vis absorption spectra of (a) AuNPs, (b) AuNPs- β -LG corona complexes (0.1 mM Au and 1 mg·mL⁻¹ β -LG) and (c) mathematical spectral overlay of AuNPs and β -LG at the same concentrations as (a) and (b).



Figure S2: RMSD of native β -LG calculated at room temperature. Error bars indicate 15 independent simulations.



Figure S3: Binding kinetics of β -LG onto AuNPs, calculated from 15 independent simulations that last 62.5 ns.



Fig. S4: Representative dynamic trajectory of interactions between β -LG and AuNPs. Highlighted regions indicate strong binding between AuNPs and β -LG with conformational changes. d_{CM}: the distance between geometric enter of β -LG and surface of AuNPs; N_c: the number of contact.



Figure S5: Colloid stability probed by the dependence of AuNPs- β -LG corona complexes absorption difference at 520 and 580 nm on β -LG concentrations at pH 4.





Figure S6: **(A)** UV-vis spectra of AuNPs- β -LG corona complexes with different β -LG concentrations. From (a) to (f), 0, 0.1, 0.2, 0.5, 1 and 2 mg/mL β -LG and 0.1 mM Au. **(B)** Enlarged plasmonic region of AuNPs from **(A)**.





Figure S7: (A) UV-vis absorption spectra of AuNPs- β -LG corona complexes at different pH and **(B)** salt concentrations.



Figure S8: SEM images of AuNPs- β -LG corona complexes. Inset shows higher magnifications.



Figure S9: Binding frequency of each amino acid residue in β -LG with AuNPs with error bars denoting the standard errors of mean (SEM). Positively charged Lys residues are highlighted by lines.



Figure S10: Representative snapshots of simulation trajectory showing the dynamic binding process in the corona formation on citrate-capped surface of AuNPs initiated from 0-62.5 ns (**a-e**). Surface bound citrates were observed to detach and replaced by β -LG which was then reoriented and eventually coupled to Au surface by the major binding site.



Figure S11: Computationally derived secondary structure alteration of β -LG upon binding to AuNPs, calculated from 1500 snapshots evenly taken at 25 ns of 15 independent simulations. The error bars correspond to the SEM estimated from independent DMD simulations of β -LG with and without AuNP binding.



Figure S12: Propensities of α -helices **(A)** and β -sheets **(B)** for each residue of β -LG. Binding with AuNPs decreases propensities of both structures (red) compared to the native state (black).



Figure S13: (A) ATR-FTIR spectra of AuNPs, β -LG and AuNPs- β -LG corona complexes (0.1 mM Au and different β -LG concentrations). Signature peaks from AuNPs and β -LG are marked in dotted red and dotted black respectively. (B) Magnification of FTIR spectrum of β -LG showing peaks of stretching vibrations of carboxylate groups from protein side chains.



Figure S14: Deconvolutions of secondary structure compositions on amide I mid-IR regions of FTIR spectra for **(A)** β -LG and **(B)** AuNPs- β -LG corona complexes. Peaks at 1632, 1639, 1651 and 1667 cm⁻¹ correspond to β -sheet, random coil, α -helix and β -turn respectively, while shoulder peaks at 1610 and 1617 cm⁻¹ are ascribed to side chains of amino acids. Some minor peak intensity and wavelength shifts can be observed and FSD was performed based on static peak positions for comparison.



Figure S15: Bright-field and wide-field 3D fluorescence surface images of AuNPs, β -LG and AuNPs- β -LG corona complexes (AuNPs=0.1 mM; β -LG=0.5 mg/mL). The meniscus of AuNPs has very low intrinsic fluorescence and β -LG fluorescence is largely quenched in the corona complexes.



Figure S16: OD_{600} values and corresponding cell numbers of a culture solution containing *E. coli* at different dilution factors of 1, 2, 4, 8, 16 and 32 with blank subtracted. No lower concentrations can be further detected.



Figure S17: Normalized UV-vis spectra of AuNPs- β -LG corona complexes in the presence of different *E. coli* concentrations with blank (no *E. coli*) subtracted. As the bacterial concentration increases, bathochromic shifts of peaks occur followed by hypsochromic shifts.



Figure S18: A scheme showing the electrostatic interactions of AuNPs- β -LG corona complexes with *E. coli*. However, other type of interactions between AuNPs- β -LG corona complexes and *E. coli* surface ligands cannot be completely ruled out.



Figure S19: RGB color profiles of AuNPs- β -LG corona complexes with various concentrations of AuNPs



Figure S20: RGB color profiles of AuNPs- β -LG corona complexes with various concentrations of β -LG.



Figure S21: Enlarged MIP CT image of GI tract at ventral view 5 h post oral administration of AuNPs- β -LG corona complexes, clearly delineating different organs in the digestive system including stomach, small and large intestines.