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Protein-biomolecule interactions play a major role in shaping corona proteome: Studies on milk interacted dietary particles

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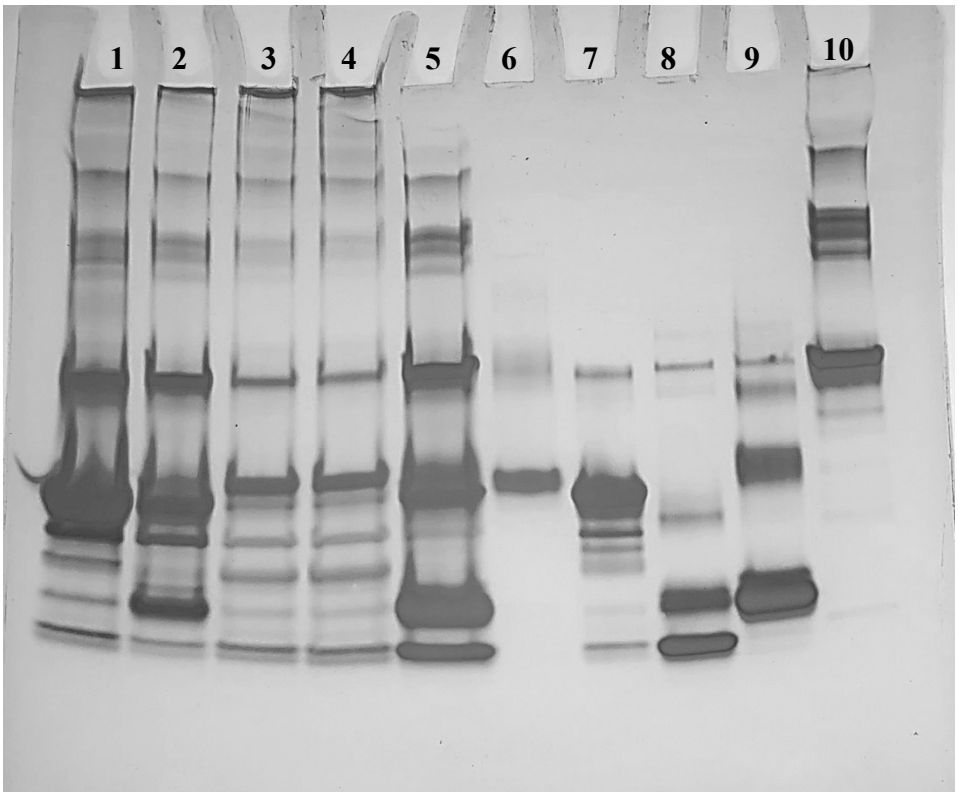
Protein preparation for one-dimension gel electrophoresis

Bovine milk protein (α-casein, β-casein, α-lactalbumin, β-lactoglobulin, bovine albumin) were selected for this experiment. One milligram per millilitre of each proteins were dissolved in PBS (pH 6.5). Two hundred microliter of each protein solution (1 $mg.mL^{-1}$) were mixed with 100 µL of dietary particles (10 $mg.mL^{-1}$) and the resulting solutions were incubated at 37°C for an hour. Controls were prepared by adding the same proportions of deionized water to the proteins mixture. After one hour incubation, the solution was centrifuged at 14,000 rpm for 15 min and the supernatant was discarded. This step was repeated twice to retain only hard corona formed on the particles.

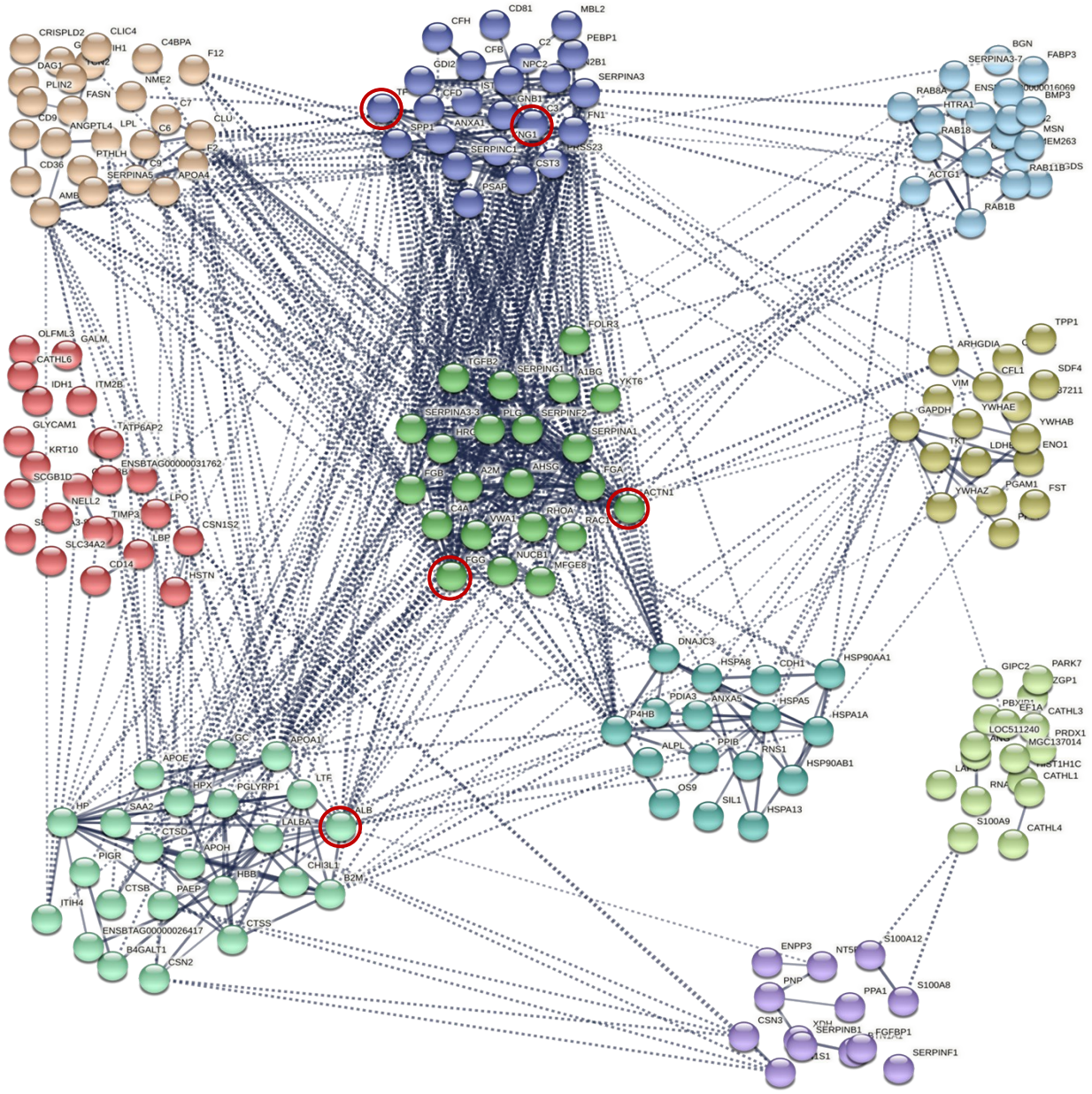
One-dimension gel electrophoresis

After the final wash of milk interacted particles, 200 μL of sample buffer containing 62.5 mM Tris-HCl, pH 6.8, 2% SDS, 25% (v/v) glycerol, 0.01% bromophenol blue and 5% β-mercaptoethanol were added to one set of particles pelleted, vortexed and boiled for 5 mins. Particles were removed from sample buffer by centrifugation at 14,000 rpm for 15 min; protein-containing sample solution was collected and transferred into a fresh tube. Twenty-five microliters of each protein-nanoparticles samples and 10 uL of each individual protein (as a control) were loaded to a sample well of a 4-20% SDS-polyacrylamide gel. The proteins were separated in the gel using 90 V for 1.5 hr in running buffer containing 2.5 mM tris, 19.2 mM glycine and 0.01% SDS. The gels were then stained in standard Coomassie blue-methanol-acetic acid solution for 30 mins at RT. The gels were subsequently washed with destaining solution (40% methanol, 10% acetic acid and 50% water) for 30 min at RT to visualize the protein bands. The gel was then stained in silver staining by following the reference method [1].

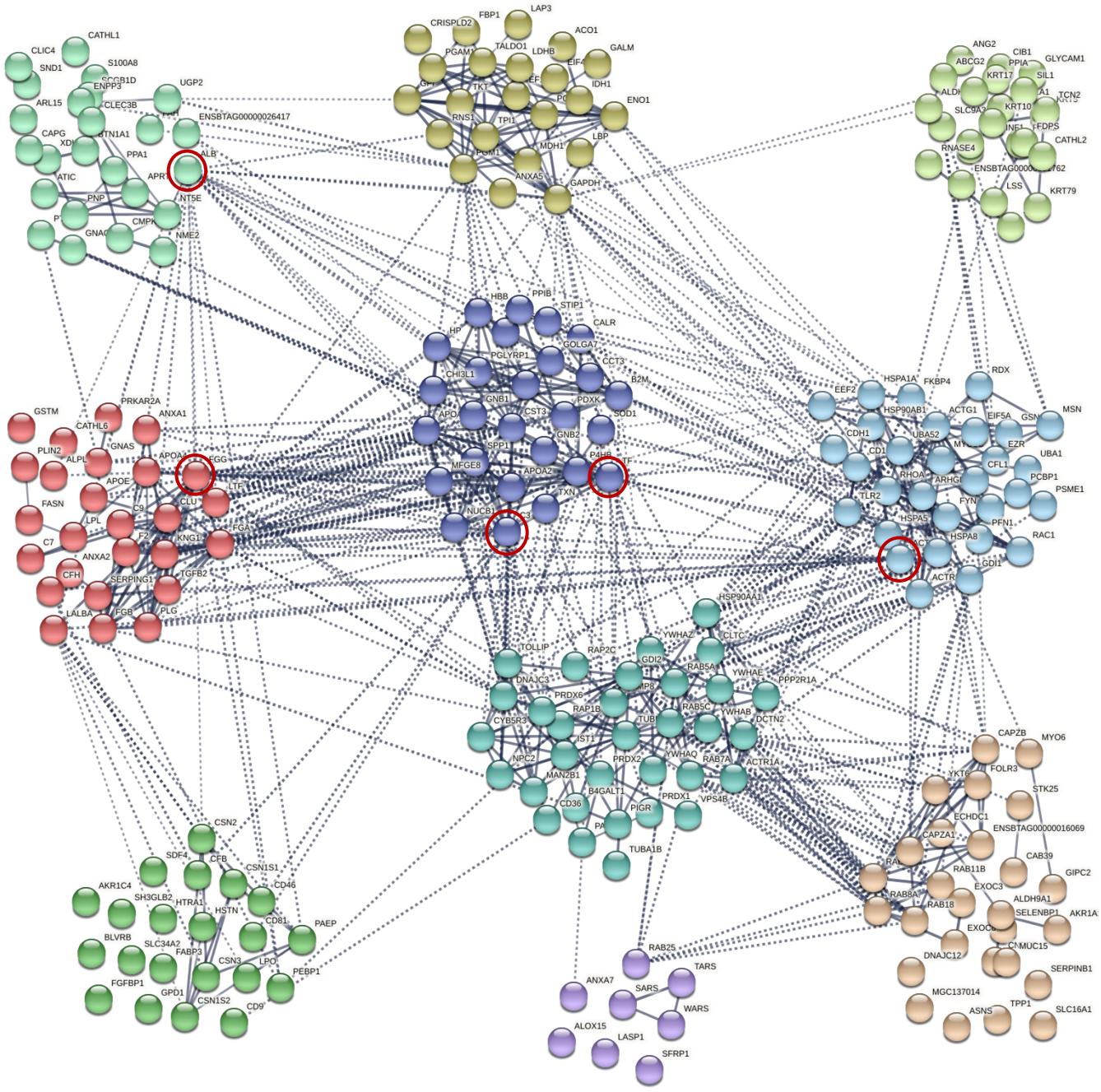
As shown in SI 1, the intensity of casein protein band were increased in silica particles adsorbed protein mixture sample. This data confirms the pattern of quantitative determination of protein adsorption onto particles given in Figure 2A.



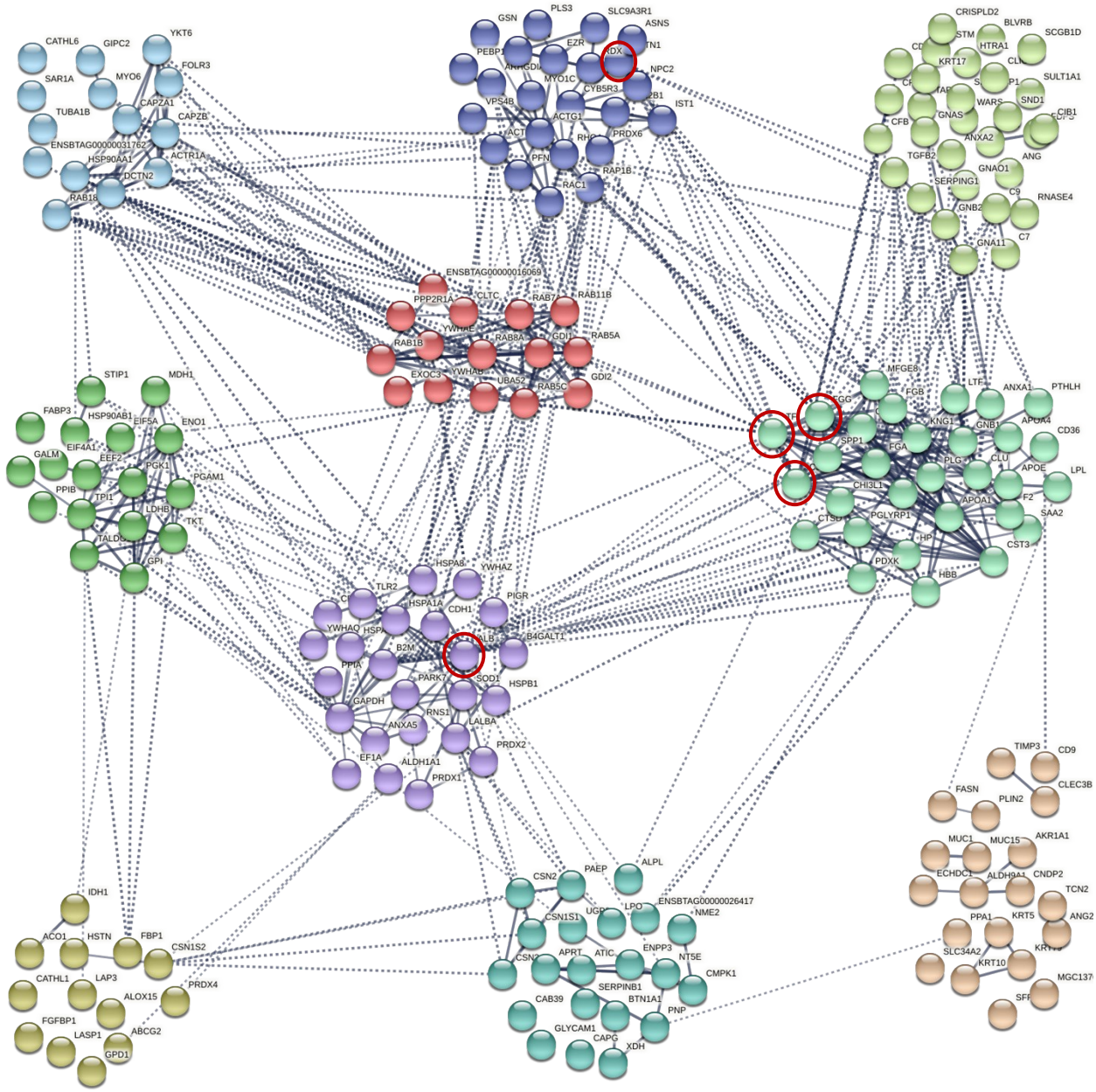
SI 1. SDS-PAGE gels of milk individual mixed proteins adsorbed on dietary particles. Lane (1) mixed proteins + FG-SiO2-NP, (2) mixed proteins + E551, (3) mixed proteins + FG-TiO2-NP, (4) mixed proteins + E171, (5) mixed proteins, (6) α-casein, (7) β-casein, (8) α-lactalbumin, (9) β-lactoglobulin, and (10) bovine serum albumin (BSA)



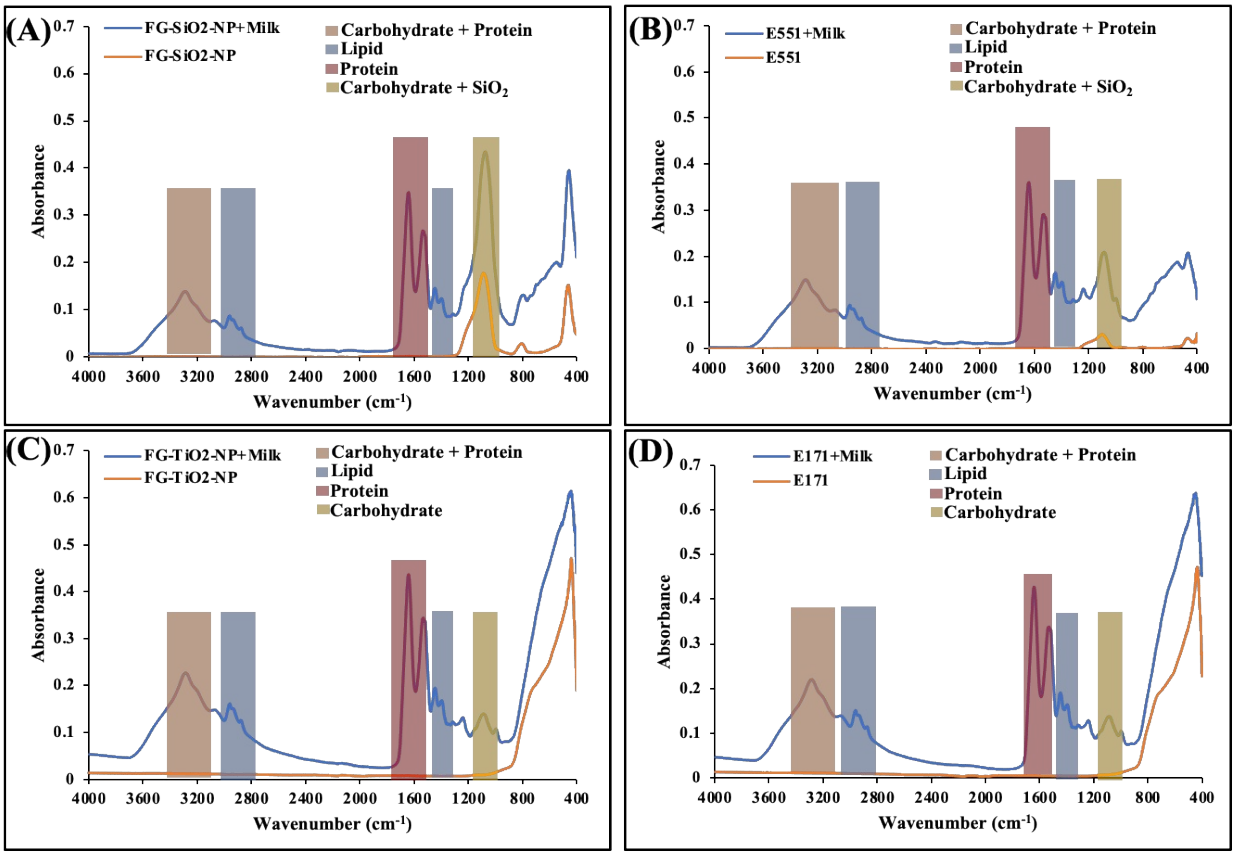
SI 2. **Protein-protein interaction network clustering with entire list of proteins** (≥ 2 no. of peptides identified in Scaffold software) on E551 identified by LC-MS/MS proteomics were developed using the STRING software. Proteins are represented as nodes and their interaction denoted through connecting lines- the thickness of which corresponds to strength of protein interaction



SI 3. Protein-protein interaction network clustering with entire list of proteins (≥ 2 no. of peptides identified in Scaffold software) on FG-TiO_2-NP identified by LC-MS/MS proteomics were developed using the STRING software. Proteins are represented as nodes and their interaction denoted through connecting lines- the thickness of which corresponds to strength of protein interaction



SI 4. Protein-protein interaction network clustering with entire list of proteins (≥ 2 no. of peptides identified in Scaffold software) on E171 identified by LC-MS/MS proteomics were developed using the STRING software. Proteins are represented as nodes and their interaction denoted through connecting lines- the thickness of which corresponds to strength of protein interaction



SI 5. FTIR spectra of milk interacted dietary particles. Food grade particles of SiO_2 and TiO_2 were interacted with skim milk for 1 hr, washed and suspended in water (10 mg.mL^{-1}), a drop of sample suspension was placed on to ATR probe, dried for 8 mins to remove water component and the spectra were taken for a wavenumber range of 4000-400 cm^{-1} using ATR-FTIR (Nicolet Summit FTIR Spectrometer, MA, USA). **(A)** Milk interacted FG-SiO_2-NP in comparison with pristine FG-SiO_2-NP, **(B)** milk interacted E551 in comparison with pristine E551, **(C)** milk interacted FG-TiO_2-NP in comparison with pristine FG-TiO_2-NP, **(D)** milk interacted E171 in comparison with pristine E171.

Peak assignments were based on published peer reviewed articles.[2,3,4] IR method have been used to determine the compositions of milk since 1964.[3] In fact, determination of major components of milk (fat, lactose and protein) using IR is now an official method.[4] The minor contributions of

proteins to absorbance at 3000-2800 cm^{-1} is negligible to obscure signal from lipid. Based on verified FTIR studies,[2] absorbance of IR in region between 1650-1540 cm^{-1} was assigned as protein peak, 1140-1020 cm^{-1} as carbohydrate, 2980-2800 cm^{-1} and 1466-1392 cm^{-1} as lipid and region between 3290-3068 cm^{-1} was assigned as protein and carbohydrate.

SI 6: Determination of presence of proteins, carbohydrates and lipids on milk interacted particles

Determination of proteins adsorbed onto particles interacted with milk

Milk interacted particles were prepared as detailed in the main manuscript. Particles were washed and suspended in water (1 $mg.mL^{-1}$) and 15 µL of the particle suspension was retrieved and mixed with 15 µL of protein eluting buffer containing 2% SDS in 20 mM sodium phosphate buffer.[5] The suspension was incubated for 1 hr at room temperature for elution of proteins from the surface of particles. The samples were centrifuged for 15 min at 20,124 g to separate the particles from the solution. Ten microliters of resulting supernatant were mixed with 100 µL of Pierce 660 containing 50 mM ionic detergent compatible reagent (IDCR).[5] After 5 min of incubation at room temperature, the absorbance was measured at 660 nm using micro plate reader (SpectraMax i3x, Multi-mode Microplate Reader, Molecular Devices, USA). The percentage of protein adsorbed onto particles was calculated based on the absorbance value determined for skim milk (not interacted with particles) using the same protocol, and the absorbance value determined for protein eluted from particle surface.

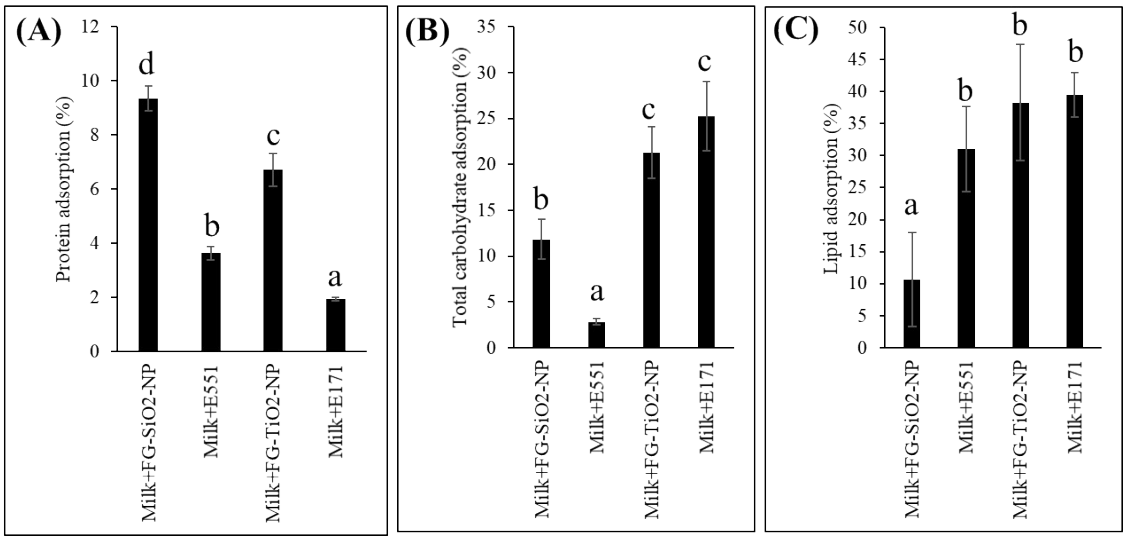
Determination of total carbohydrates adsorbed onto particles interacted with milk

Total carbohydrate present on particles interacted with milk was determined using phenol-sulfuric acid method.[6] For this, milk interacted particles were washed and suspended in water (1 $mg.mL^{-1}$) and 50 µL of particle suspensions were added to wells in a 96 well plate. Subsequently, 150 µL of concentrated H_2SO_4 and 30 µL of 5% phenol in distilled water were added. The plate was incubated in 5 min at 90°C, followed by cooling to room temperature. The absorbance was measured at 490

nm. The percentage of carbohydrate adsorbed onto particles was calculated based on absorbance value determined in skim milk (not interacted with particles) using the same protocol, and the absorbance value in the particle suspension.

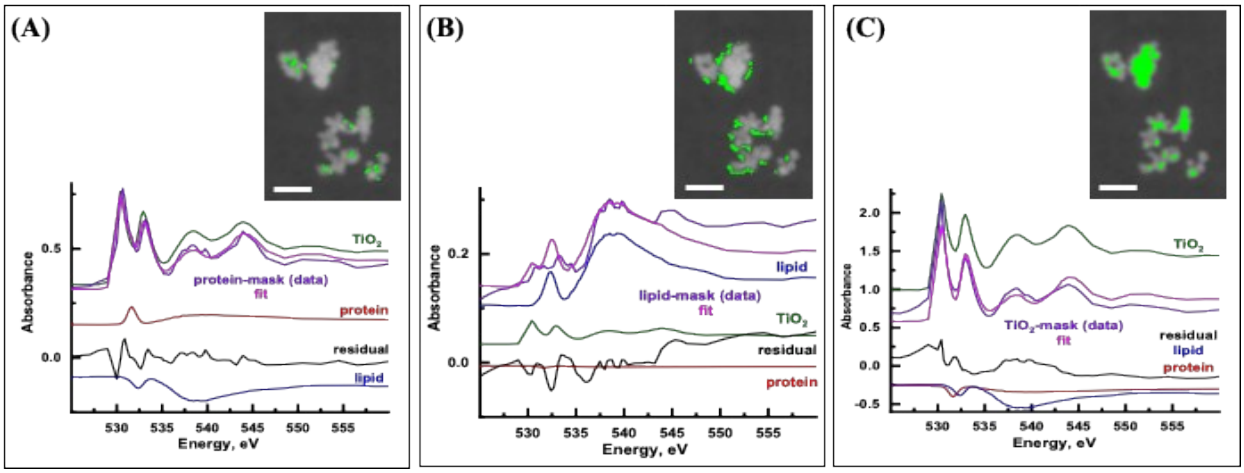
Determination of total lipids adsorbed onto particles interacted with milk

Total lipids present on particles interacted with milk was determined using Sudan black B staining, according to protocol reported but with modification.[7] Sudan-black B staining solution was prepared by adding 500 mg of Sudan-black B, 20 mL of acetone, 15 mL of acetic acid and 85 mL of water. The dye solution was stirred for 30 mins at room temperature and undissolved dye was removed by centrifugation at 20,124 g for 15 min. Five hundred microliters of milk interacted particle suspensions (1 $mg.mL^{-1}$) were mixed with 500 µL of dye solution and incubated for 30 min at RT. Subsequently, the samples were centrifuged at 20,124 g for 15 mins to collect the stained total lipid and washed with water for 3 times. The dye on particles was redissolved in 200 µL of acetone and the suspension was centrifuged at 20,124 g for 15 min to remove particles from the solution. Ten microliters of supernatant were mixed with 190 µL of water and the absorbance was measured at 600 nm. The percentage of lipids adsorbed onto particles was calculated based on absorbance value determined in skim milk (not interacted with particles) using the same protocol, and the absorbance value of dye eluted from particles.



SI 6. Quantification of the presence of proteins, carbohydrates and lipids in the surface corona of dietary particles. (A) The presence of proteins adsorbed onto milk interacted particles were confirmed through Pierce 660 as detailed. (B) the presence of carbohydrate in the surface corona was confirmed by Phenol-sulphuric acid method, (C) the percentage of lipids in the surface corona was confirmed by Sudan-black B staining method. Average values are plotted in the graph and the different letters indicate significant differences (Duncan, $p<0.05$).

Our studies confirmed the presence of proteins, carbohydrates and lipids (which may contain triglycerides, fatty acids, phospholipids, sterols, lipo-proteins, fat soluble vitamins, etc). There was difference among particles for the relative amount of proteins, carbohydrates and lipids in surface corona. Generally, the presence of carbohydrates and lipids were higher in particles of TiO_2 in comparison to SiO_2 and for proteins vice versa. Total fat present in skim milk is in the range of 0.04-0.3% and it is composed of triacylglycerols, diacylglycerols, monoacylglycerols, free fatty acids, phospholipids and sterols and the ratio of phospholipids to total fat is relatively higher in skim milk.[8] The presence of fat soluble vitamins (Vitamin A and Vitamin D) may also contribute to the total lipids determined using Sudan Black B staining.



SI 7. Linear combination fitting of the derived spectra using the reference spectra from Figure 6. (A) Protein derived, **(B)** lipid derived, and **(C)** TiO_2 derived spectra. The derived spectra were determined by threshold masking of the pixels with high intensity from their respective component map. The insert shows the location of the pixels (green) on the average image of the O K-edge stack.

Table SI 1. Amount of protein, lipid and TiO_2 in the spectra derived by threshold masking of the pixels with high intensity from their respective component map as determined by linear combination fitting using the reference spectra from Figure 6.

Component	Thickness (nm) of components from the derived spectra		
	Protein	Lipid	TiO_2
Protein[a]	158	-6.50	-274
Lipid[b]	-119	143	-331
TiO_2	58.3	5.90	171
Chi-squared	0.051	0.042	0.74

a - albumin, b - 1,2-dipalmitoyl-sn-glycero-3-phosphocholine

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